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REGENERATION OF DEFECTS IN THE CALVARIA

AN EXPERIMENTAL STUDY

BY

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PREFACE

The theme of this study was suggested to me by the pioneer of Finnish pediatric surgery, Dr. Matti Sulamaa, M.D., Chief Surgeon of the Children's Hospital of Helsinki University. His keen eye for the interesting facts in his surroundings, his constructive criticism, his grasp of fundamentals, and his infectious enthusiasm have been of support to me throughout the course of my work. His advice has been of inestimable value. For all this I am deeply in his debt.

I offer my warm thanks to the Chief of the Children's Hospital, Professor Niilo Hallman, M.D., for his unfailing interest in my work, for his kindness in granting me permission to work in the animal laboratory of the hospital and for his helpful assistance whenever I needed it.

I thank the Chief of the Finnish Red Cross Hospital, Professor Aarno Snellman, M.D., for the support he has given me. As the greatest authority in the field of neurosurgery in Finland, he has offered many helpful comments on my work. My thanks are also due to Dr. Lauri Laitinen, M.D., of the same hospital, whose valuable study on craniosynostosis is related to the present thesis, for his critical perusal of my work and for much encouragement.

I wish to express my gratitude to Professor William Kerppola, M.D., Chief of the First Medical Department of the University Central Hospital, Helsinki, for the interest he has shown in my work. I am indebted to him for granting me facilities for housing some of my experimental animals in the animal room of the above hospital. As an experienced scientist he has given me the benefit of his views on scientific research in general.

I express my sincere thanks to Miss Ulla Bauer for her valuable advice on histological techniques. I am also grateful for the skilled assistance of Miss Aino Toivonen, M.Ph., and Mr. Paavo Korhonen,

who did the photographic work at the Institute of Photography of Helsinki University; the former was responsible for the radiograms and the latter for the microphotographs.

I thank all those of my friends and my colleagues who in one way or other assisted me in my work.

Helsinki, February 1960.

Kari Sirola.

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INTRODUCTION

In various textbooks of surgery, neurosurgery and pathology and in monographs dealing with bone tissue there are statements to the effect that the regenerative capacity of the bones of the calvaria or cranial vault is poor. Thus fissured fractures of the skull heal considerably slower than fractures of other bones. It is a general belief that bone defects of the cranial vault in adult human beings never ossify. This belief is also supported by the large number of different plastic operations which have been developed for the repair of cranial defects.

At the Children's Hospital, University of Helsinki, observations on the regeneration of cranial defects at variance with the above-mentioned opinion have been made in connection with surgery on children and particularly on infants. In 1953, an osteoplastic operation on a girl with congenital defect of the sternum and with the heart, covered by the membraneous pericardium alone, protruding through this opening (ectopia cordis) was performed by Dr. M. Sulamaa, Chief Surgeon of the Hospital. At the first operation, which was carried out at the age of 2 months, a bony transplant, 7 by 4 cm in size, was taken from the left parietal bone. A second operation was done when the patient was 6 months old and then a bony plate, 6 by 4 cm in size, was removed from the right parietal bone. Part of the outer periosteum was left *in situ* and the dura mater was not injured. The operation was successful. On palpation the defects in the cranial vault felt hard within a couple of months and about 2 years after the operation the parietal bones appeared in radiograms to be normal with the exception of a slight irregularity of the bony structure at the sites of operation. At palpation the parietal bones seemed quite firm but on the right side at the site of the trephination there was a shallow depression in the bone. This depression was still demonstrable

5 years after operation. Its floor felt bony and could not be perforated with a needle.

In the surgical treatment of subdural haematomata of newborn babies and infants at the Children's Hospital, limited local trephination either alone or in conjunction with wide osteoplastic opening of the skull and radical removal of the haematoma membranes was performed. Follow-up examination of 29 such patients was carried out, the time of observation ranging between 6 months and 9 years. In 7 cases no trace of the trephination, or only insignificant roughness of the bone, was observed in the radiograms. These patients had been operated on at the age of 1 to 6 months (1 ¼ months on average) and the time of observation was 2 to 7 years. In all other cases, in which the age at operation was ½ to 10 months (3 months on average), some traces of the operation were seen in the radiograms. If an osteoplastic opening had been made, the borders between the bony flap and the surrounding bone were found to have disappeared within 2 years. Traces of drilling or trephination were observed as much as 9 years after operation although the skull had been operated on at an early age. In these cases the bone defects in the radiograms had ragged and blurred edges. In many cases it was possible to palpate a shallow depression with a firm floor in the surface of the skull. Attempts made in some cases to push a needle through the bony floor of such a depression were unsuccessful. It is possible that in all cases the floor of the opening had ossified even though the defect could still be traced in the radiogram.

These observations from the field of pediatric surgery show that the cranial vault of infants, at any rate, possesses a considerable regenerative capacity. A perusal of the literature on the regeneration of the calvarial bones together with the clinical results mentioned above prompted the carrying out of the animal experiments presented in the following.

HISTORICAL SURVEY

FRACTURES OF THE SKULL IN PREHISTORIC TIME

Since the earliest time man has realized the effectiveness of a blow on the head when fighting against an animal or another man. In some *skulls of prehistoric animals* traces of fractures have been observed which have possibly been caused by man. Cleland [25] investigated a number of skulls of the giant cave bear in which there was a ragged hole on the right side. He assumed these to have been caused by the hafted poleaxe of Neanderthal man. A cave drawing in Moravia shows a cave bear with its head injured [1]. These injuries of the skull have generally been splintered or depressed fractures. Whether the injury was fatal or whether the animal survived has been determined by examination of the margins of the fracture defect. In the former case the defect remained unchanged with sharp edges [31], but if the animal survived the blow the bony edges of the opening became rounded or there was regenerative proliferation [31, 110, 146]. In some cases changes in the bone have been observed which have been attributed to infection [119].

In *skulls of prehistoric man* found in different parts of the world fractures have often been observed. The opinion has been expressed that Neanderthal man [29], and Sinanthropus Pekinensis [163] smashed the heads of their enemies in order to eat the brains. Broca [17] described the skull of an adult Cro-Magnon woman in the left frontal bone of which there was a crack 5 cm long and 1½ cm wide. Eversion of the margins of the hole and radiating cracks could be observed. The wound, which had obviously been inflicted with an axe, had probably penetrated to the brain and had suppurated. Lartet and Chaplain-Duparc [92] have likewise

described the skull of a Cro-Magnon man in which there was a patent defect with rounded edges in the right parietal region. In the Berlin museum Cleland [25] examined a Neolithic skull in the crown of which there was an irregular defect with rounded edges and old fracture lines around it. The author presumed this to be an extensive comminuted fracture in which removal of bone splinters and resection of the edges may possibly have been carried out as a surgical measure. On the other hand, skulls have also been found in which there were fracture defects with no changes in the edges and in these cases death was considered to have followed immediately upon the injury [109].

SIGNS OF REGENERATION IN TREPHINED SKULLS OF PREHISTORIC MAN

Trephination is the oldest known surgical procedure. Burial findings prove that it was carried out in the Neolithic period in many parts of Europe [103, 138], in the pre-Columbian time in Peru [136, 139, 156], since early times in Central America, Mexico and North America [27, 102] and on the islands of the South Pacific even before the arrival there of Europeans [18, 19, 30, 32, 40, 42, 106]. Investigators of these trephined skulls have been interested in the extent to which the patients survived the procedure. This has been estimated on the basis of the bone edges: If they were rounded or if there was proliferation, the patient is believed to have lived for a considerable period after the operation. Openings observed in old skulls have also sometimes erroneously been interpreted as produced by surgical measures [144].

Regenerative changes have been particularly investigated in the Inca skulls. Of these, Tello [156] examined 400 trephined skulls collected by Ales Hrdlicka, finding evident cicatrization in 62 per cent or 250 cases. Stewart [153] examined 75 of these ancient Indian skulls with trephine holes found in Peru and preserved in the U.S. National Museum. He observed signs of regeneration in 30 of them. He described the degree of healing of the bone by the expressions »advanced stage of healing», »a healed opening» and »a completely healed opening». »Healing», as used by him, means rounding of the margins of the trephine opening and slight prolifera-

Fig. 1. — Trephined skulls from ancient Peru. By courtesy of Dr. T. D. Stewart and the Bulletin of the History of Medicine.

tion, but in a photograph there is an example of a case of »complete healing» or almost complete ossification of the defect (see uppermost fig. 1). All the skulls found belonged to adults. In 24 cases (or 32 per cent) there were porous changes over a rather large well-limited area, the shape of a geometric polygon (sometimes square) in the outer surface of the bone surrounding the trephination defect. Stewart considered these to be the result of postoperative infection or of antiseptic and irritative substances used in the treatment of the wound. They indicate that before operation a comparatively large piece of skin was removed from the site of the operation, thus leaving it open. The Incas opened the skull in three principal ways [139, 153]. The commonest method was to saw off a square piece of the calvaria. Another was to make a circular trephine opening. The third and rarest method was to drill holes in the bone near one another in a circle and thereafter to remove the central disc. In 21 out of Stewart's 75 cases, regeneration had made it impossible to decide what method of trephination had been used. In many Peruvian skulls two, and sometimes even four or five, trephine openings have been observed which have often been at different stages of healing [54, 135, 167]. The majority of these trephinations were probably carried out at a time when the Inca culture was at its height, *i.e.* about 1100—1500 A.D. (cf. Karsten [82]) but it has also been suggested that the oldest trephined skulls date from as early as 3000 B.C. [139].

According to extant hieroglyphic writings, trephination of the skull as a method of surgical treatment was not carried out in ancient Egypt [16], yet one trephined skull, thought to have belonged to one of the high priests of Amon, has been preserved from this time. The hole drilled in the frontal bone was 2 cm in diameter and Courville [28] described it as follows: »The bone of this opening shows evidence of absorption indicating that the High Priest survived his injury for a considerable period of time».

In the archipelago of the South Pacific, magicians have performed trephination of the skull as a therapeutic measure up to the present century [18, 19, 30, 32, 40, 42, 106]. In connection with the finding of such skulls the results of these operations have likewise been estimated from the »regeneration» of the edges of the opening in the bone [120, 121, 135], but I have found no detailed descriptions of the changes in the bone at the site of trephination.

In prehistoric skulls found in different parts of the world various signs of regeneration of the bones of the calvaria have been noted. The majority of the findings show that the opening made in the skull remained persistently patent even though a rounding of the bony edges and possibly some amount of proliferation of the bone had taken place. In some skulls, however, and particularly in those from ancient Peru, almost complete ossification of an earlier defect has been observed. On the basis of these findings, and in face of general medical opinion, S. L. Rogers [136] stated that complete ossification of a trephine opening must in some cases be considered possible. Daland [34] described a Peruvian skull in which there was an evident depressed fracture which had healed spontaneously. Whether the defects in question might originally have been depressed fractures or whether trephination had originally been carried out at an early age in such regenerated cases are problems which have not received sufficient attention in the literature.

INFORMATION IN THE MEDICAL LITERATURE ON THE REGENERATIVE CAPACITY OF THE HUMAN CALVARIA

The oldest extant medical text is an ancient Egyptian hieroglyphic writing discovered by Edwin Smith and probably dating from the period between 3000 and 2500 B.C [16]. Here the conservative treatment of various injuries of the skull is described in detail but there is no mention of the healing of the cranial bones. Neither did Hippocrates [69] (460—377 B.C.) make any mention of this point although he was well acquainted with trephination as a method of treatment of injuries of the skull. In the writings of the Roman Celsus [24] (about 30 B.C.) we find the following slightly obscure sentence which describes the postoperative development of a trephine opening of the skull: »When things are going well, flesh grows up from the membrane (dura) itself and from the bone as well if it is in two layers, so that the space between the bones becomes filled up; sometimes it even grows out above the skull». It is probable that »flesh» here refers to the cicatricial granulation tissue at the site of operation.

Heine [65] (1837) was probably the first to publish the observation

that the capacity of the cranial bones to form new bone is very much poorer than that of the long bones, for instance. According to him, defects in the human calvaria remain patent although they may become reduced in size from the edges and the sharp edges become rounded. It was his opinion that the new bone originated in the open diploic space. In 1887, Göz [53] suggested that the regenerative capacity of the bones of the calvaria was greater than had hitherto been presumed. He described some adult cases in which after trephination a considerable capacity for regeneration was noted after a longer period of observation. In a 41-year-old woman, in particular, autopsy showed that an opening 8 by 10 cm in size made in the calvaria because of osteomyelitis had ossified completely during the course of 26 years.

Examples similar to those of Göz were later presented, demonstrating that defects of the calvaria may ossify [9, 104, 134] provided that the dura has not been injured. Matti [104] was of the opinion that small openings in the calvaria ossify, while those over 5 cm in diameter generally do not. He also referred to the fact that in children regeneration is better than in adults.

Others have stressed the low regenerative capacity of the bones of the cranial vault [62, 87, 99, 154]. According to Brüning's [21] clinical observations artefact defects of the skull only very rarely heal spontaneously. The author believed that reports to the contrary might possibly be erroneous: the defect is covered by firm connective tissue and radiological and patho-anatomical examinations in this field had not been carried out to a sufficient extent.

Since the end of the last century a large number of methods for the closure of calvarial defects have been presented, bone, cartilage, metal or some alloplastic material being used [7, 45, 55, 101, 105, 123, 124, 125, 130, 143]. The abundance of methods reflects the assumption that openings in the calvaria do not ossify spontaneously. Closure of trephine openings even in children has been recommended [150], particularly if the defect is in the frontal region [78].

The adoption of osteoplastic craniotomy (Wagner [158], 1889), together with a general recognition of the importance of asepsis in surgical operations, meant a large step forward in neurosurgery and solved the problem of closure of trephine openings. Opinions diverged as to whether the bone plate detached from the calvaria survived or not. Despite arguments to the contrary [36], it was concluded that

the cut-off piece of bone dies when reimplanted but is slowly replaced by new bone (»restitutio ad integrum«) [4, 12].

Many authors have traced the healing of different types of fractures of the skull on the basis of radiograms [48, 49, 59, 71, 74, 75, 79, 80, 99]. It has been established that a narrow fissure disappears within about 1½ years, while a wider crack may be observed for a period of 4 to 5 years, and should the diastasis be wider than 4 mm the fracture line probably never disappears [75]. Simultaneous lesion of the dura may further reduce the rate of healing of a skull fracture [79, 104]. It is unanimously agreed that the regenerative capacity of depressed fractures is good whether the fragments are elevated to their proper site or not. Cranial fissures ossify first on the dural side [46, 93, 104]. In connection with bone fractures exostotic bone ridges may form at the margins of the fractured bone on the inner surface [46, 104]. On the basis of their radiological investigations Glaser and Blaine [49] state: »In operative defects wherein the bone has been removed, or in cases of depressed fracture, wherein the fragments have been removed, the cranial defect never becomes smaller, the only change being a rounding of the edges«. If osteoplastic craniotomy is carried out on an infant, Glaser and Blaine's experience is that the bone heals completely. On the other hand they presented a case in which after osteoplastic trephination performed on a 28-year-old man the bony plate became largely resorbed during the course of 7½ years. A similar case had previously been described by Moullin [114]. Many authors have stated that the regenerative capacity of the cranial bones is greater in children than in adults [38, 46, 56, 57, 71, 76, 137, 144, 145, 169]. According to Moorhead and Weller [111], surgical repair of defects of the cranial bones in children is therefore not called for.

In the literature I have not found conclusive evidence of the healing of actual cranial defects in children as compared with adults. It is not an easy matter to collect patho-anatomical series with a long enough period of regeneration. When cranial defects are evaluated on the basis of radiograms it should be remembered that in plain film one cranial wall is always projected against the other. It is then not possible to assert with confidence [64] whether there is a total defect or one in which the floor is wholly or partly ossified (as stated by Lauche [93], for instance). Practical experiences in the field of neurosurgery show that needles can be introduced

into the brain chambers through drill holes in the adult calvaria years after their formation [149]. On the basis of the observations mentioned in the beginning of this thesis and of information obtained from the literature [46, 111] there is reason to assume that trephine openings in the skulls of infants, at any rate, may ossify completely.

EARLIER EXPERIMENTAL INVESTIGATIONS

It might be presumed that during the long history of trephination the question of the healing of openings in the bones of the calvaria would now and then have aroused interest. Yet comparatively few experimental investigations in this field can be found in which this particular problem constituted the main focus of interest. However, observations that incidentally throw light on this question have been made in connection with other studies on such subjects as the repair of defects with some artificial material, craniosynostosis and otosclerosis.

The first investigation on the regenerative capacity of the calvaria was probably that of Heine [65], who stated that the normal structure of the bones and sutures is re-established after total or partial removal of the calvaria in young or adult animals. Berezowsky [9] trephined holes 6 mm in diameter in the skulls of 5 rabbits and found that the openings were covered by new bone within a month provided the dura mater had been left intact, but remained entirely patent if the dura was removed. Defects 3 to 6 cm wide made in the calvaria of young dogs seemed to heal less well. By these experiments he proved that for the healing of a trephine opening in the bone the dura mater is an essential factor. In Germany, Kochiyama [85] published a thesis in which he described the different histological stages in the regeneration of the calvaria on the basis of a study of 11 rabbits. He mentioned that he had not found any similar investigation in the earlier literature. In 5 mm wide trephine openings he observed connective tissue formation and regeneration of the bone from the margins. Ossification was almost complete 106 days after the operation. Principally from the diploë, but to some extent also from the outer periosteum and the strip of dura at the bone margins, the bone grew into the

connective tissue occupying the opening in the shape of osteoblast-covered bridges. Troitzky [157] investigated the growth of the skull and factors influencing its shape. He described different modes of trephination which he carried out on guinea-pigs, rabbits and dogs. He came to the following conclusions: 1) If the dura is removed from the area of the bone defect, or if it is injured by scraping, regeneration of the bone will not take place in this area. 2) If the dura is preserved, both bone and sutures will be regenerated in the original sites; thus the sutures constitute topographic borders across which bone cannot grow. 3) In adult animals defects made in the calvaria do not ossify. The latter conclusion, however, is based on only one experiment made on a 10-month-old guinea-pig. Sutro and Jacobson [155] trephined an opening about 3 cm in size into both parietal bones of 27 young and adult rats. They found that after 5 months the defects in the young rats were totally ossified. In the adult animals ossification was slower and in some of them the defect covered by a connective tissue membrane still persisted after 5 months. Pritchard [126] observed that cuts sawn in the rat calvaria and through the dura had not yet ossified after 3½ months. Bourne [14] drilled holes 2 mm in diameter into the parietal bones of guinea-pigs (nothing is mentioned with regard to the dura) and similar holes into their femurs. The healing of the former was considerably slower. From 45-day-old rats Walker [159] removed the centre of the cranial vault, the corresponding dural area and the sagittal sinus. He believed that the opening was first covered by a connective tissue membrane which, with the exception of the sutures, ossified completely within 20 days. In a clinical case, an infant, Löer and Sanders [100] had observed marked regeneration in a postosteomyelitic defect in the calvaria and they carried out experiments on 6 young rabbits. They found, as had already been pointed out by Simpson *et al.* [147], that separate bone islands had formed on the dural surface at the site of the trephination. If in connection with trephination the dura was painted with a silver nitrate solution, regeneration did not take place and the dura adhered to the brain surface. They were of the opinion that the dura mater is of paramount importance in regeneration.

When towards the end of the last century, osteoplastic craniotomy had been introduced, some investigators became interested in the regeneration of the skull after reimplantation of the bone

plate, and animal experiments were undertaken to throw light upon this question. Contrary to what had earlier been believed [168], Barth [4] and Biagi [12] were of the opinion that the piece of bone reimplanted into the trephine opening dies and is gradually replaced by new bone which seems principally to grow on the inner surface. From observations on his control series Biagi thought that without reimplantation of the bone the defect becomes covered principally by connective tissue (in rabbits). Experimental trephinations have also been carried out in investigations on the suitability of various alloplastic materials for the repair of cranial defects. Campbell *et al.* [23] trephined openings 2 to 3 cm in diameter in the skulls of 42 dogs, covering the defects with ticonium or vitallium plates. After several months (12 to 15 months even), the defects were covered by firm fibrotic tissue; a little new bone was, however, observed in some defects, mostly at the margins. Using 11 cats, Pudenz [130] investigated the suitability of tantalum for the repair of a trephine opening. As a secondary observation he mentioned that under the plate (*i.e.* on the dural surface) grew a bony layer which entirely covered the 1.5 by 2.5 cm wide opening after 10 months. Beck *et al.* [8] made similar observations on dogs, using an acrylic resin disc for the repair of the opening.

Ingraham *et al.* [77] investigated the problem of craniosynostosis, cutting channels in the cranial vault of Rhesus monkeys and dogs. These saw cuts healed either partly or totally within the course of 100 days. Regeneration was particularly rapid in young animals. Moss [112] proved Troitzky's statement on the topographic stability of the sutures to be inexact. He removed from a young rat a piece of bone and a corresponding piece of the dura adjoining a suture and showed that when the animal grew the suture margin at this site was displaced in the direction of the trephine opening. The defect remained patent.

In investigations on otosclerosis some authors have discussed the problem of how, after fenestration, the opening drilled into the bone plate could be kept patent. Lempert *et al.* [94] carried out a histological investigation on the regeneration in connection with non-penetrating defects made with different instruments in the skulls of 12 cats. In these cavities bone formation took place in the same way independently of the nature of the instrument used. They believed that new bone is formed when fibroblasts are trans-

formed into osteoblasts. Holmgren *et al.*[70] carried out similar experiments in rabbits. Guggenheim and Guggenheim[58] drilled small holes in the skulls of rabbits and found that these holes remained patent if painted with a solution of silver nitrate or trichloro-acetic acid.

In the investigations referred to above the influence of the dura mater or the inner periosteum of the calvaria on the formation of new bone has been stressed. Likewise reference has been made to the better regenerative capacity of a young animal as compared with an adult or old animal. The concept of the regeneration of the calvaria that we obtain from the observations made is not quite consistent, however, as has appeared from the views cited above. To clarify this concept, a more thorough investigation of this phenomenon on the basis of new research was considered desirable.

PROBLEMS

1. To what extent do trephine openings in the calvaria regenerate in experimental animals?
2. What course does regeneration follow?
3. On what local factors does regeneration depend?
4. Can a difference in the regeneration of the calvaria be demonstrated between animals of different ages?

MATERIAL AND METHODS

The experimental animals were 83 rabbits and 8 cats. The youngest rabbits were 4 days old and the oldest 2 to 3 years old. Of the cats, 5 were 1- to 6-week-old kittens and 3 were adult animals. The newborn animals were anaesthetized by hypothermy with ice while Nembutal 33 mg/kg was given intraperitoneally, intramuscularly or intravenously to older animals together with ether inhalation. The scalp of the head was shaved and the operative field sterilized with alcohol and a 1 per cent mercurochrome solution, after which the field was further covered with a layer of Nobecutan. The calvaria was exposed through a midline sagittal incision. In the commonest operation, which in the following will be called »standard trephination», the outer periosteum or pericranium was resected from the trephination area and bone removed from the area of the parietal and frontal bones injury to the dura mater being avoided as far as possible (fig. 2).

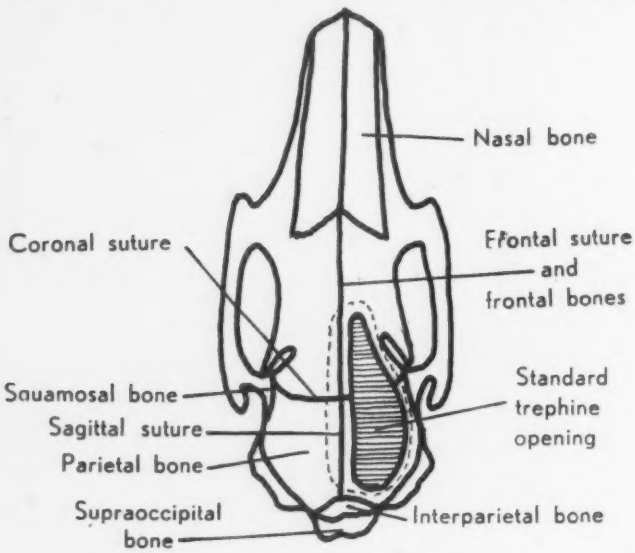


Fig. 2. — Schematic picture of the rabbit skull from above. The hatching in the area of the right parietal and frontal bones indicates the site from which the bone was generally removed (standard trephination). The dotted line indicates the area of resection of the pericranium.

With a blunt scalpel a succession of holes was drilled around a small area, the bone was removed from this area and, the dura being detached with a blunt probe, the opening in the bone was enlarged with small scissors or bone forceps. On the medial side of the opening the pericranium was resected slightly farther than elsewhere; it was divided near the sagittal suture on the opposite side (fig. 2). The length of the opening was usually 15—34 mm and its greatest width (in a transverse direction) 5 to 14 mm. It was not always possible to carry out trephination on rabbits entirely without injury to the dura. Every now and then the dura was injured, mostly at the medial bone margin near the anterior fontanelle, sometimes also at the posterior end of the trephine opening, near the posterior fontanelle. The wound was closed with interrupted silk sutures and covered with a Nobecutan film. In 6 cases »calvariectomy» was carried out, the opening in the bone comprising a continuous area on either side of the calvaria, leaving the dura mater and the superior sagittal sinus *in situ* (fig. 3). The follow-up time of the rabbits varied from 3 days to 6 months and of the cats from 4 to 5 months. The animals were killed either with ether or, in most cases, with intravenous injections of air. The calvaria was sawn off and examined and general autopsy was carried out. The bone

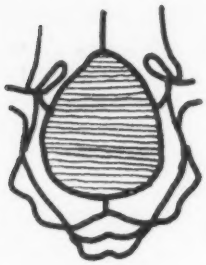


Fig. 3. – Calvariectomy.

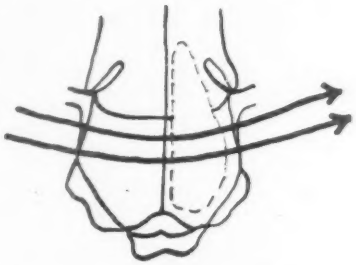


Fig. 4. — Histological samples were cut transversely across the calvaria; they comprised the area between the arrows.

specimens were kept in a 10 per cent formalin solution. Radiograms of these were taken with a special biological X-ray apparatus ERK 10/50 (made by Messrs. Oy. Havemann Ab., Helsinki). The photographic data were as follows: 20 kW, 10mA, 20 sec., focal distance 30 cm. The films (Ilford Ilfex) were developed in Final developer (Agfa) for a period of 4 minutes at +20°C.[1] After decalcification, a narrow piece was cut off transversely across the calvaria on the posterior side of the coronary suture (fig. 4). In some cases a sample of the coronary suture was also taken from the regenerated (right) side. The bone specimens were stained according to Weigert-van Gieson's method for microscopic examination. The material was divided into six groups (A—F, see table). The rabbits have been

[1] The radiograms printed in this paper are positives.

referred to groups A—E according to the operative procedure used in each case; the cats belong to group F. The operative methods are described in greater detail in the next section.

All animals were included in the series except those few which died during or soon after the operation. Radiograms were taken of 65 of the rabbits and of all the cats. In the following cases no radiograms are available: R 1, R 2, R 4, R 7, R 9, R 10, R 14, R 17, R 18, R 20, R 21, R 27, R 37, R 46, R 50, R 68, R 72, R 83. Histological examinations were not made of the cats or of the following rabbits: R 7, R 19, R 21, R 37, R 42, R 46, R 50, R 68, R 72. The whole heads of the rabbits R 37, R 50 and R 72 were boiled, and the skulls cleaned and examined macroscopically. Detailed case histories of all experimental animals as well as the radiograms are filed in the archives of the Children's Hospital, University of Helsinki.

RESULTS

The results were evaluated on the basis of macroscopic inspection, radiograms (negatives) and histological specimens. When compared, the radiograms and the histological bone specimens, which were cut transversely across the entire calvaria about 5 mm posteriorly from the coronary suture, revealed the same details, such as sutures, unossified connective tissue areas and separate bone plaques. The radiograms illustrated the structure and »radiological thickness» of the bone in various areas. In the histological specimens, in addition to the microscopic details, the linear thickness of the bone could be estimated.

TABLE

GROUP A

(rabbits)

Case No.	Age	Weight at Operation	Operation	Size of the Opening	Post-operative Period	Weight at Sacrifice	Result
R 1	4 days	77 g	Standard trephination	15×5 mm	2 wks.	200 g	Defective closure of the opening. Slight cerebro-dural attachment.
R 2	4 days	95 g	Calvariectomy	15×11 mm	2 wks.	110 g	Death by wound infection. No regeneration.
R 3	4 days	76 g	Standard trephination	15×5 mm	1 mo.	665 g	Defective closure. A narrow cerebro-dural attachment.
R 4	4 days	80 g	Standard trephination	15×6 mm	1 ½ mo.	775 g	Almost no regeneration. Vast lesion of the dura, strong attachment to the cerebral cortex.
R 5	4 days	80 g	Standard trephination	15×6 mm	4 mo.	1580 g	A solitary defect, 8×3 mm, cerebral attachment. Thick bone elsewhere.
R 6	8 days	150 g	Standard trephination	20×10 mm	2 mo.	1120 g	Defective closure. Cerebral attachment.
R 7	2 wks.	145 g	Calvariectomy	25×15 mm	11 days	120 g	Death by wound infection. No regeneration.
R 8	2 wks.	155 g	Standard trephination	18×9 mm	3 wks.	685 g	Defective closure. Some cerebro-dural attachment.
R 9	2 wks.	200 g	Standard trephination	20×9 mm	1 ½ mo.	870 g	Small defects. Slight cerebro-dural adhesions.
R 10	2 wks.	300 g	Standard trephination	13×6 mm	7 wks.	800 g	Defective closure, islets of new bone.
R 11	2 wks.	220 g	Standard trephination	20×9 mm	2 mo.	950 g	Defective closure. Cerebro-dural attachment.
R 12	3 wks.	307 g	Standard trephination	20×8 mm	2 wks.	630 g	Bony islands in the lateral region of the opening.
R 13	3 wks.	310 g	Standard trephination	20×8 mm	1 mo.	1240 g	Defective closure.
R 14	3 wks.	130 g	Standard trephination	18×8 mm	1 ½ mo.	510 g	Defective closure. Slight adhesions to the cerebral cortex.
R 15	3 wks.	120 g	Standard trephination	21×9 mm	7 wks.	600 g	Defective closure.
R 16	3 wks.	130 g	Calvariectomy	22×17 mm	3 mo.	1270 g	Defective closure.
R 17	1 mo.	620 g	Standard trephination	20×5 mm	1 mo.	1050 g	A sagittal crack of 2 mm between the plates of new bone. Most bone on the lateral side.
R 18	1 mo.	700 g	Standard trephination	20×5 mm	2 mo.	1850 g	Convulsions — death. A narrow sagittal crack of fibrous tissue, otherwise completely closed. Most bone on the lateral side.
R 19	1 mo.	620 g	Standard trephination	20×4 mm	4 mo.	2810 g	Defective closure.
R 20	5 wks.	240 g	Standard trephination	20×6 mm	2 mo.	930 g	Thick new bone, 2 small defects.
R 21	5 wks.	210 g	Standard trephination	22×8 mm	2 mo.	830 g	Complete closure.

R 22	1 ½ mo.	550 g	Standard trephination	28 × 12 mm	2 wks.	780 g	Layer of fine granules of new bone.
R 23	1 ½ mo.	680 g	Standard trephination	32 × 12 mm	1 mo.	1360 g	Complete closure, except 2 minute defects. Thin layer of new bone.
R 24	1 ½ mo.	510 g	Standard trephination	32 × 12 mm	1 mo.	420 g	Anomaly of the incisors — starvation — death. Defective closure, thin islets of new bone.
R 25	1 ½ mo.	670 g	Standard trephination with large resection of the peri-cranium	30 × 11 mm	2 mo.	2020 g	Complete closure by thin bone.
R 26	1 ½ mo.	560 g	Standard trephination	21 × 10 mm	2 mo.	1620 g	Complete closure. The new bone slightly thinner than normal.
R 27	1 ½ mo.	280 g	Standard trephination	25 × 8 mm	3 mo.	1650 g	Completely closed by a thick layer of bone.
R 28	1 ½ mo.	750 g	Calvariectomy	30 × 20 mm	3 mo.	2870 g	Closed except for a 2 mm crack in the median line. Uneven bone.
R 29	7 wks.	1280 g	Standard trephination	25 × 13 mm	8 days	1180 g	Osteogenesis beginning (microsc.).
R 30	7 wks.	660 g	Standard trephination	28 × 13 mm	8 days	790 g	Osteogenesis beginning, fine granules of new bone.
R 31	7 wks.	560 g	Standard trephination	26 × 13 mm	3 wks.	820 g	Granules of new bone.
R 32	7 wks.	1080 g	Standard trephination	25 × 13 mm	2 mo.	1920 g	Complete closure by a layer of bone slightly thinner than normal. A minute defect corresponding to a small dural lesion.
R 33	7 wks.	910 g	Standard trephination	32 × 10 mm	4 mo.	2830 g	Complete closure, thick bone.
R 34	2 mo.	640 g	Standard trephination	29 × 10 mm	3 days	650 g	Callus formation beginning (microsc.).
R 35	2 mo.	660 g	Standard trephination	28 × 12 mm	5 days	740 g	Osteogenesis beginning (microsc.).
R 36	2 mo.	1340 g	Standard trephination	28 × 13 mm	2 mo.	1950 g	Complete closure, fairly thick bone.
R 37	2 mo.	1120 g	Standard trephination	33 × 10 mm	4 mo.	2880 g	Complete closure, thick bone.
R 38	2 mo.	1120 g	Standard trephination	30 × 10 mm	4 mo.	2530 g	Complete closure. The new bone slightly thinner than normal.
R 39	2 mo.	1420 g	Standard trephination	30 × 12 mm	5 mo.	3120 g	Complete closure. The new bone in places thinner than normal.
R 40	9 wks.	1280 g	Standard trephination	30 × 9 mm	4 mo.	3000 g	Complete closure. A thin area in the parietal region.
R 41	9 wks.	1400 g	Calvariectomy	30 × 18 mm	5 mo.	2980 g	Complete closure. The middle part, 11 × 7 mm, closed by separate bone plaques.
R 42	2 ½ mo.	1430 g	Standard trephination	30 × 12 mm	4 mo.	2490 g	Complete closure. Parietal bone slightly thinner than normal.
R 43	2 ½ mo.	1170 g	Standard trephination	16 × 7 mm	4 mo.	2620 g	Complete closure. The new bone slightly thinner than normal.
R 44	3 mo.	2300 g	Standard trephination	30 × 11 mm	1 mo.	2780 g	Incomplete closure. Thin islets of new bone.

Continued

Case No.	Age	Weight at Operation	Operation	Size of the Opening	Post-operative Period	Weight at Sacrifice	Result
R 45	3 mo.	1970 g	Standard trephination	19×10 mm	2 mo.	2700 g	New bone chiefly on the lateral part. Thin bone plaques all over the opening. Incomplete closure.
R 46	Adult	3820 g	Standard trephination	19×8 mm	2 wks.	2920 g	Death (diarrhoea?). No wound infection. No macroscopic regeneration.
R 47	Adult	3000 g	Standard trephination	31×10 mm	2 mo.	2850 g	Incomplete closure. Bone plaques considerably thinner than the surrounding bone. Margins of the trephine opening distinct in x-ray.
R 48	Adult	3150 g	Standard trephination	29×11 mm	2 mo.	3550 g	Most of the opening covered with new bone which is thin as compared with the surroundings. Some smaller defects left. Incomplete closure. Margins of the trephine opening distinct in x-ray.
R 49	Adult	3550 g	Trephination on the parietal bone	17×10 mm	2 mo.	3780 g	Incomplete closure. New bone much thinner than normal. Margins of the trephine opening clear in x-ray.
R 50	Adult	2910 g	Standard trephination	33×12 mm	4 mo.	2960 g	Incomplete closure, uneven bone islets.
R 51	Adult	2450 g	Trephination of a round opening on the parietal bone	5×5 mm	4 mo.	2960 g	Defective closure. Decrease of the diam. to 3 mm and some very delicate and minute bone plates in the middle (x-ray).
R 52	Adult	4030 g	Standard trephination	34×14 mm	4 mo.	4050 g	Complete closure except 2—3 small defects. Borders of the opening clear in x-ray. The new bone considerably thinner than normal.
R 53	Adult	3250 g	Trephination on the parietal bone as far as the coronal suture	16×10 mm	4 mo.	4000 g	Almost complete closure by bone islands of diff. sizes. New bone much thinner than normal. Margins of the trephine opening clear in x-ray.
R 54	Adult	4030 g	Standard trephination	19×6 mm	5 mo.	4050 g	New bone on the margins. Total defect of 10×4 mm.
R 55	Adult	2550 g	Standard trephination	31×11 mm	5 mo.	2730 g	Incomplete closure. New bone on the margins, coarse bone granules in the middle. A total defect of 7×2 mm.
R 56	Adult	2850 g	Standard trephination	30×11 mm	6 mo.	3280 g	Almost complete closure. Relatively thick bone centrally, thin and defective bone anteriorly and posteriorly.

GROUP B

(rabbits)

R 57	2 mo.	1000 g	Standard trephination, pericranium replaced	28 × 10 mm	1 mo.	730 g	Diseased animal (intracran. haematoma, infection?). Complete closure except for one small defect. Thin bone.
R 58	2 mo.	970 g	Standard trephination, pericranium replaced	30 × 13 mm	3 mo.	1600 g	Complete closure, except for a minute defect at the coronal suture. A thin area in the posterior part.
R 59	1 ½ mo.	590 g	Standard trephination, pericranium replaced	24 × 9 mm	3 mo.	1910 g	Complete closure except for a minute defect at the coronal suture. New bone slightly thinner than normal.
R 60	2 mo.	840 g	Standard trephination, pericranium replaced	27 × 11 mm	3 ½ mo.	2140 g	Complete closure except for 2 minute defects. New bone slightly thinner than normal.
R 61	1 ½ mo.	650 g	Standard trephination, pericranium replaced	29 × 10 mm	4 mo.	2150 g	Complete closure except for one minute defect corresponding to a small lesion of the dura. Thick bone with normal structure.
R 62	1 ½ mo.	630 g	Standard trephination, pericranium replaced	30 × 13 mm	4 mo.	2330 g	Complete closure with bone of normal structure.

GROUP C

(rabbits)

R 63	4 days	60 g	Standard trephination, dura covered with p. film	17 × 6 mm	4 ½ mo.	1870 g	Complete closure by thick bone chiefly under the film. A thin, incomplete bridge of bone over the film. Coronal suture incompletely re-established (x-ray).
R 64	3 wks.	160 g	Standard trephination, dura covered with p. film	22 × 7 mm	4 mo.	1580 g	Thick bone under the film. Incomplete closure. Coronal suture not re-established.
R 65	3 wks.	160 g	Standard trephination, dura covered with p. film	21 × 7 mm	4 mo.	2050 g	Uneven bone layer under the film, incomplete closure. Coronal suture incompletely re-established (x-ray).
R 66	1 mo.	330 g	Standard trephination, dura covered with p. film	22 × 7 mm	3 mo.	1620 g	Nearly complete closure by new bone under the film. New bone growth laterally also above the film. Coronal suture not re-established (x-ray).
R 67	5 wks.	350 g	Standard trephination, dura covered with p. film	22 × 8 mm	3 ½ mo.	1530 g	Complete closure by new bone under the film, except for a minute defect at the coronal suture. The coronal suture curves anteriorly (x-ray).

Continued

Case No.	Age	Weight at Operation	Operation	Size of the Opening	Post-operative Period	Weight at Sacrifice	Result
R 68	1 ½ mo.	600 g	Standard trephination, dura covered with p. film	22 × 5 mm	1 mo.	?	Death from unknown cause.
R 69	1 ½ mo.	560 g	Standard trephination, dura covered with p. film	20 × 5 mm	3 mo.	1870 g	Thick bone under the film. Almost complete closure. Coronal suture not re-established (x-ray).
R 70	7 wks.	940 g	Standard trephination, dura covered with p. film	22 × 9 mm	4 mo.	2500 g	Thick uneven bone under the film. A small defect in the ant. part. Coronal suture not re-established (x-ray).
R 71	3 mo.	1550 g	Standard trephination, dura covered with p. film	25 × 8 mm	2 mo.	2250 g	New bone under the film. Incomplete closure. Coronal suture incompletely re-established? (x-ray).
R 72	Adult	2800 g	Standard trephination, dura covered with p. film in the ant. part of the opening	31 × 10 mm	4 mo.	3300 g	Incomplete closure. The new bone under the film. Coronal suture not re-established (macrosc.).
R 73	1 ½ mo.	610 g	Standard trephination, p. film on the dura. Pericranium replaced	26 × 9 mm	2 mo.	1380 g	Anomaly of the incisors, relative undernourishment. Thick bone under the film, complete closure except for one minute defect at the coronal suture. Thin bone brim above the film, size of the aperture 22 × 6 mm. Coronal suture not re-established (x-ray).
R 74	1 ½ mo.	600 g	Standard trephination, p.film on the dura. Pericranium replaced	20 × 7 mm	6 mo,	3020 g	The calvaria completely re-established (x-ray) except for the lacking right coronal suture. Under the film (micr.): the bone plate thick medially, thin laterally. Above the film: complete bridging of bone, thin medially, thicker laterally.

GROUP D

GROUP D

(rabbits)

R 75	1 ½ mo.	470 g	Standard trephination. 3 mm of the lateral bone edge covered with a polythene strip	25 × 11 mm	3 ½ mo.	2360 g	A small bone defect beside the film strip. Another minute defect at the coronal suture corresponding to a small dural lesion. Otherwise normal regeneration.
R 76	1 ½ mo.	590 g	Standard trephination. The lateral bone edge covered with a polythene strip	25 × 7 mm	3 mo.	2160 g	Complete closure except the poor bone formation beside the film strip.
R 77	1 ½ mo.	900 g	Standard trephination. Polythene strips fixed on all bony edges	23 × 5 mm	3 mo.	2060 g	The edges of the trephine opening clear-cut. Fairly thick bone plates in the opening.
R 78	1 ½ mo.	850 g	Calvariectomy. All the bone edges covered with polythene strips	22 × 18 mm	3 mo.	2070 g	The edges clear-cut. Bone islets at the periphery of the opening, none in the middle. By growth the films a little drawn apart in the lateral corners.

GROUP E

(rabbits)

R 79	1 ½ mo.	1020 g	Standard trephination, dura excised.	18 × 5 mm	3 mo.	2160 g	The opening covered by a thin fibrous membrane. Rounding of the bony edges. Strong cerebral attachment.
R 80	1 ½ mo.	710 g	Standard trephination, dura excised, the opening covered with pericranium	20 × 6 mm	3 mo.	1820 g	Rounding of the bony edges. No regeneration except for a solitary plate connected with the ant. margin of the opening. Cerebral attachment on the margins.
R 81	1 ½ mo.	950 g	Standard trephination with excision of the dura. Dural defect covered with polythene film. Pericranium replaced	17 × 6 mm	3 mo.	2180 g	Rounding of the edges. No regeneration except for a solitary bone plate under the polythene connected with the post.-medial margin. Slight cerebral attachment on the margins.
R 82	1 ½ mo.	780 g	Standard trephination with excision of the dura. Dural defect covered with polythene film. Pericranium replaced	21 × 5 mm	3 mo.	1420 g	Rounding of the edges with minute bony protuberances and one larger bone plate under the polythene connected with the medial margin. A thin fibrous membrane elsewhere. No cerebral attachment.
R 83	Adult	2950 g	Trephination on the parietal bone. Dura excised	15 × 10 mm	2 mo.	3030 g	Rounding of the bony edges and reduction of the opening to 10 × 6 mm. Strong cerebral attachment.

GROUP F

(cats)

Case No.	Age	Weight at Operation	Operation	Size of the Opening	Post-operative Period	Weight at Sacrifice	Result
C 1	6 days	160 g	Standard trephination	25 × 12 mm	4 mo.	2070 g	Complete closure. Margins of the trephine opening not discernible in x-ray. The new bone slightly thinner than normal.
C 2	17 days	195 g	Trephination on the parietal bone	19 × 9 mm	4 mo.	1750 g	Incomplete closure. Margins of the former opening not discernible in x-ray.
C 3	17 days	240 g	Standard trephination	33 × 12 mm	4 mo.	2000 g	Almost complete closure; an irregular narrow defect near the coronal suture. Margins of the former opening not discernible in x-ray.
C 4	17 days	275 g	Trephination on the parietal bone as far as the coronal suture	20 × 10 mm	4 mo.	1230 g	Incomplete closure. The margins of the trephine opening not distinguishable in x-ray.
C 5	1 ½ mo.	570 g	Standard trephination	35 × 17 mm	4 mo.	1560 g	Incomplete closure by ridges and islets of new bone. The margins of the opening not distinguishable in x-ray. Approx. ½ of the surface of the opening covered by thick membrane only.
C 6	Adult	3290 g	Standard trephination	33 × 12 mm	4 mo.	4000 g	Defective closure. New bone at the margins and 2 bone islets centrally are considerably thinner than the surroundings. The old margins ill-defined in x-ray.
C 7	Adult	2930 g	Trephination on the parietal bone	19 × 9 mm	4 mo.	3880 g	Defective closure: thin bone on the margins. Limits of the former opening still discernible in x-ray.
C 8	Adult	5120 g	Trephination on the parietal bone	20 × 12 mm	5 mo.	4100 g	Incomplete closure. New bone considerably thinner than the surroundings. The rounded margins of the trephine opening still visible in x-ray.

GROUP A

This group comprised 56 rabbits of varying age, on the majority of which trephination with removal of the outer periosteum was carried out in the right fronto-parietal region, the dura mater being preserved (standard trephination, fig. 2). From 5 animals a wide continuous piece of bone was removed from the middle of the calvaria (calvariectomy, fig. 3).

In all animals of this group, regeneration of the bone defect was observed, repair taking place in different ways, depending on age.

In rabbits under 1½ months old, regeneration was incomplete. In almost all these cases entirely unossified areas of varying size persisted (fig. 5). The brain surface often adhered to these defects over a small area; brain tissue adherent to the dural surface could also often be seen in the histological specimens. In all radiograms

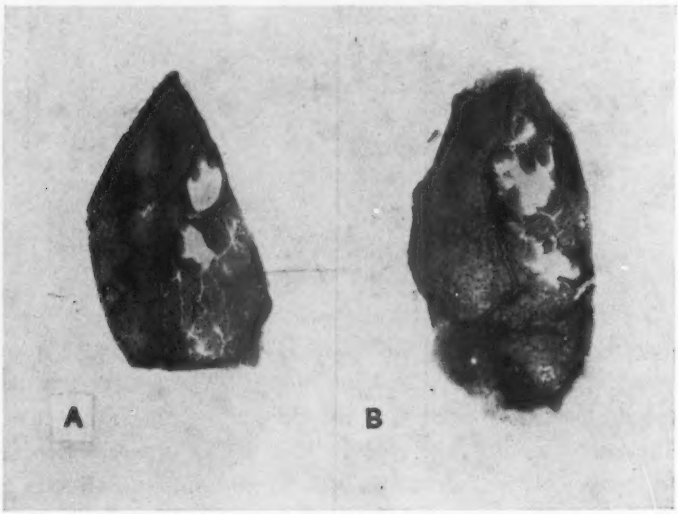


Fig. 5. — Rabbits of the youngest age group, in which regeneration was incomplete. *A*. Radiogram of case R 6. The operation was carried out at the age of 8 days and the observation time was 2 months. *a*. The corresponding histological specimen. In the trephination area there is an ossification defect of connective tissue which also adheres to the cerebral cortex. *B*. Radiogram of case R 11. The operation was carried out when the rabbit was 2 weeks old and the observation time was 2 months. *b*. The corresponding histological specimen. In this specimen also, a defect filled with connective tissue can be observed in the bony bridge, to the inner surface of which brain tissue adheres. *b'*. Magnification of the area outlined in the former picture. × 40.

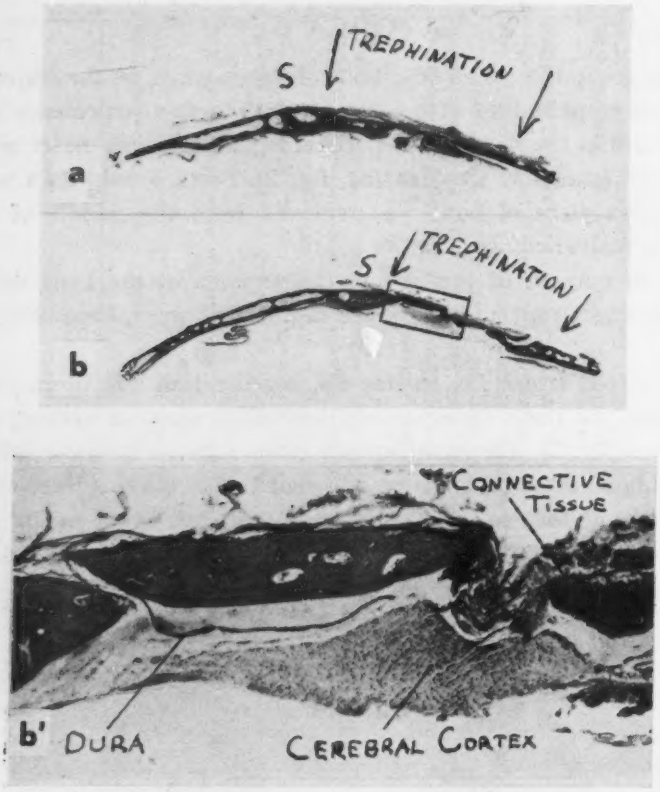


Fig. 5 a, b, b'.

the contours of the trephine openings had disappeared. The new bone could often be seen as isolated islands of varying size or as processes extending from the bone margin. The longer the period of regeneration, the coarser the diploic structure. Microscopic examination demonstrated that in this age group remodelling of the bony edges of the defect was best. It should be mentioned that the rabbits of this age group were the first to be operated on. It might be thought that lack of operative experience somewhat influenced the result in these cases. It is my belief, however, that any such influence was small, since in all cases of the present material the operative technique was essentially the same.

As for the remodelling of the edges in animal R 12, for instance, which had been operated on at the age of 3 weeks, a piece of dead bone could be clearly seen after 2 weeks within the living bone tissue in the region of

both the medial and the lateral bone margins. In animal R 13, which had also been operated on at the age of 3 weeks, no traces of necrotic bone could be found in the specimen after 1 month. With the exception of a few cases in which, after 1 to 2 months, some insignificant piece of old bone matrix was found within the new bone at a site corresponding to the margin of the former defect, remodelling of the bony margins was completed in animals of this age group during the course of a postoperative period of 1 to 4 months.

In *1½-to 3-month-old rabbits* regeneration was throughout good (fig. 6). The best picture of the course of regeneration is obtained from a study of this age group (see page 59). Macroscopically the bone could be seen to have grown so as to be almost normal within 4 months. Radiologically the contours of the defect had disappeared as early as 2 months after the operation. In some cases there were quite small pin-size ossification defects corresponding to small

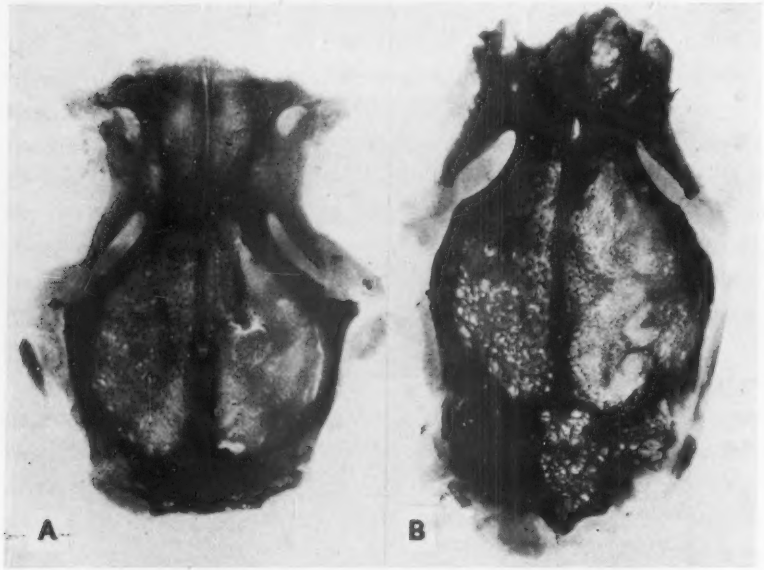
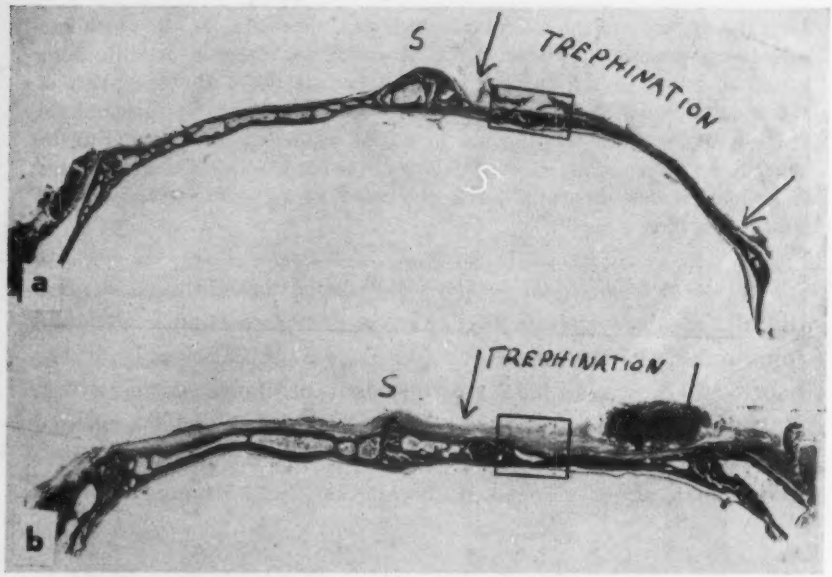


Fig. 6. — Rabbits of the middle age group (1 ½ to 3 months) *A*. Case R 32 in which operation was carried out at the age of 7 weeks, 2 months after operation. Note the minute ossification defects near the anterior and posterior fontanelles. *a*. The corresponding histological specimen. *a'*. Magnification of the area outlined in the former picture. × 40. *B*. Rabbit R 38 trephined at the age of 2 months, 4 months after operation. Suture formations are present both at the site of the right coronary suture and elsewhere in the new bone. *b*. The corresponding histological specimen. *b'*. Magnification of the area outlined in the former picture. A secondary suture can be seen to the left in the picture. × 40.



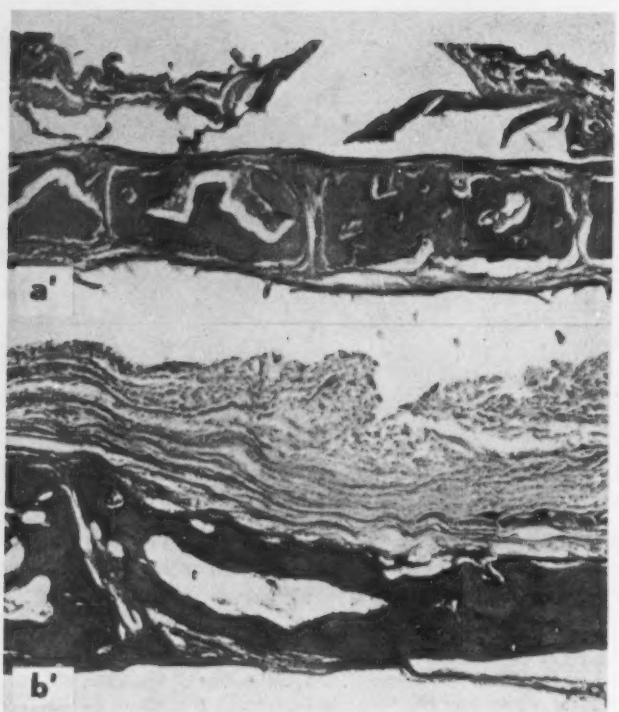


Fig. 6 a, b, a', b'.

lesions of the dura produced at operation. Small defects of this kind mostly occurred in the region of the coronary suture near the midline, *i.e.* near the anterior fontanelle. No adhesions between the dura and the brain surface were observed. In the regenerated region the course of the coronary suture could in most cases be established radiologically. The suture was often rather irregular, however, and not as tortuous as the normal suture. Moreover, it was often somewhat displaced. In the new bone there were, in addition, many other suture-like formations running in various directions which bordered and joined together separate bone areas of varying size. These secondary sutures, too, were less tortuous than normal sutures. In their microscopic structure, the secondary sutures did not differ basically from normal ones (fig. 22). They consisted of rather dense connective tissue which, without any margin, joined the fibrous layer of the pericranium on one side and that of the dura on the other. In the suture tissue bordering the bone margins, which consisted of primitive bone, numerous osteoblasts with large nuclei were seen. After 2 months of regeneration, the radiograms began to show diploë formation which gradually became coarser and grew more and more to resemble the structure of the surrounding bone. However, 4 months after operation the diploë structure was not yet fully developed microscopically and there was little normal bone marrow tissue. In this group the remodelling of the bone edges was practically complete after 4 months but in many cases the dead bone was resorbed within 2 months.

In adult animals of uncertain age, bone regeneration of varying degree was observed in all cases (fig. 7). It sometimes happened that the trephine opening was covered by a thin bone layer 2 months after operation. Four months after operation the trephine defect was radiologically still clearly distinguishable from the surrounding bone. Macroscopically, the inner surface of the regenerated bone was smooth and merged into the surrounding bone without demarcation. In the new bone layer there were numerous connective tissue sutures and, as compared with the former group, there were more ossification defects in the adult rabbits. No adhesions were observed between the brain surface and the dura. The microscopic specimens showed that the regenerated bone was strikingly thin as compared with the surrounding bone. Otherwise the new bone

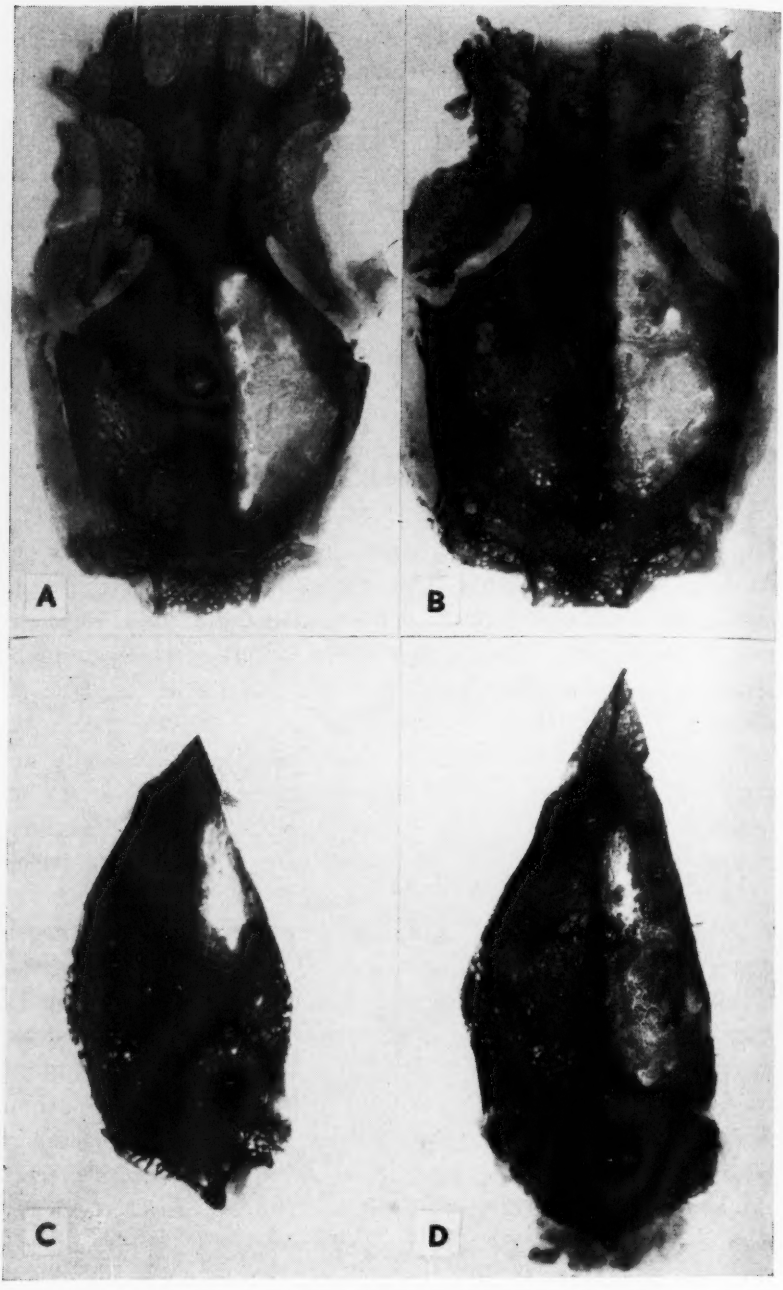


Fig. 7 A, B, C, D.

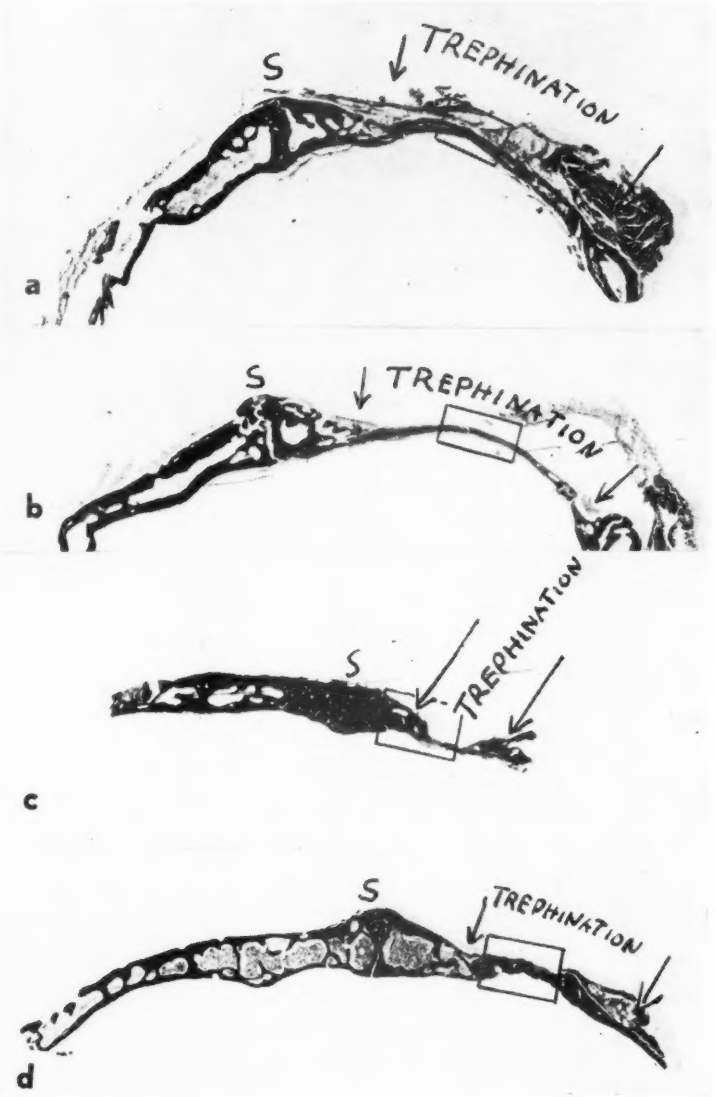


Fig. 7. — The group of adult rabbits. *A*. Rabbit R 48 2 months after operation The margins of the trephine opening are well defined in the radiogram but on the dural surface there is a thin, fairly continuous bone layer in which there are, however, some small defects. *B*. Rabbit R 52 4 months after trephination. The defect is covered by a comparatively continuous, very thin bone layer. The outlines of the original defect are distinctly seen. *C*. Rabbit R 54 5 months after operation. A complete ossification defect 10 by 4 mm wide can be seen. New bone has formed at the margins of the defect to a width of some millimetres. *D*. Rabbit R 55 5 months after trephination. In the anterior part of the trephine opening there is an ossification defect and in the centre of the

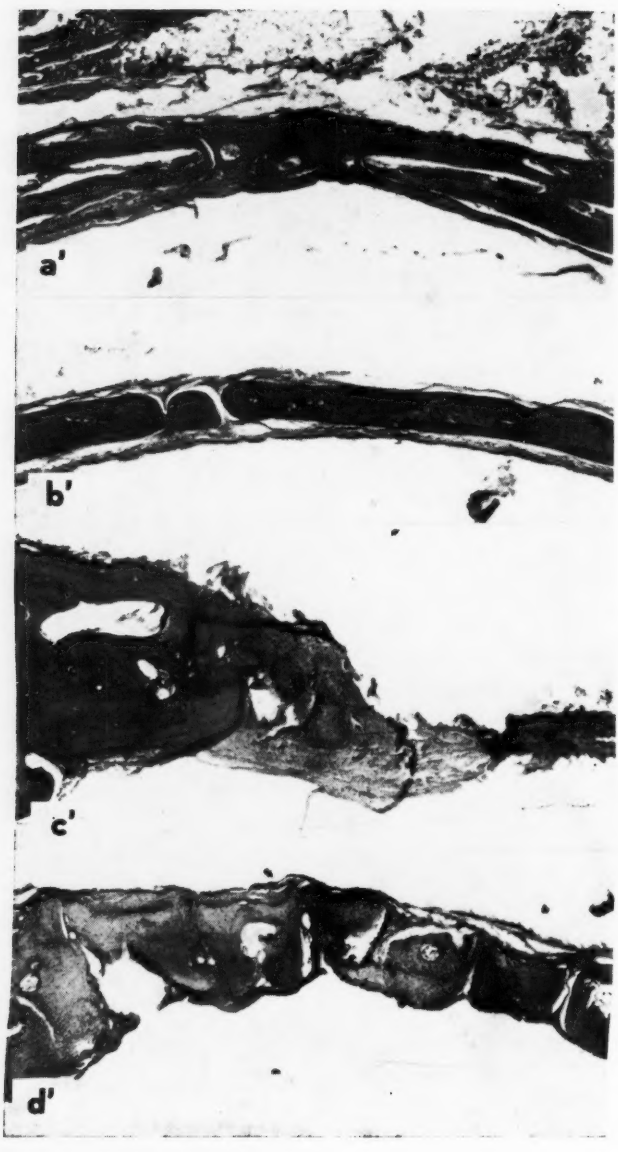


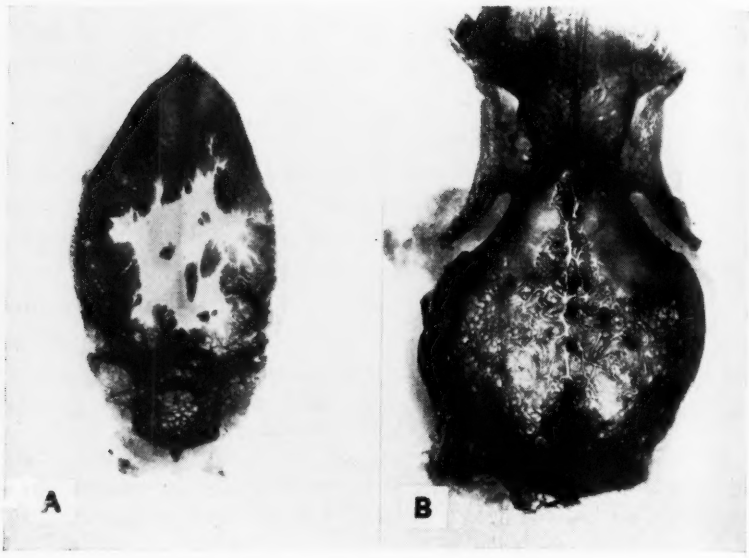
Fig. 7 a', b', c', d'.

posterior part innumerable quite small isolated bone islands have formed. At the margins continuous bone has formed. The original opening is of approximately the same size as in the two upper figures (R 48 and R 52). *a*, *b*, *c* and *d* are the corresponding histological specimens and *a'*, *b'*, *c'* and *d'* section enlargements (× 40). Note that in fig. *b* the regenerated bone layer is without diploë and thinner than in fig. *a*.

tissue was basically of the same kind as in the former age group. Two months after trephination, the bone was partly lamellated but in some places (at the sites of growth) there was quite primitive bone (woven bone) and outside it osteoblasts with large nuclei. Four months after trephination, bone marrow could also be observed here and there in the diploic space of the new bone. In the group of adult rabbits, the dead bone areas on the margins of the trephine opening were resorbed remarkably slowly. In these areas, particularly in the region of the medial edge, bone matrix (fig. 23) without nuclei could be clearly seen as late as 5 to 6 months after operation, and here and there through the matrix there penetrated blood vessel canals formed by new bone.

In one adult rabbit (R 54, fig. 7 C, c, c') 5 months after trephination there remained a quite patent ossification defect 10 mm long and 4 mm wide; the original size of the defect was 19 by 6 mm. In another case (R 55, fig. 7 D, d, d') in which the original defect had been 31 by 11 mm wide, 5 months after trephination the defect was largely covered by small bone granules and in the frontal region there was a completely patent ossification defect 7 by 2 mm wide. In an animal (R 56) which was killed 6 months after operation, the defect was almost completely ossified but here and there the new bone layer was so thin that it was possible to perforate it with a needle between the bone plaques. In case R 51 a 5 mm wide opening was made in the middle of the parietal bone. In 4 months a thin strip of bone 1 mm in width grew from the bone margin on the inner side. The rest of the opening seemed to be covered by stiff connective tissue only, which could easily be pierced with a needle, but the radiogram showed some minute extremely thin bone plaques at this site.

Of the 5 rabbits of different ages on which trephination extending from side to side across the sagittal suture (*calvariectomy*) had been carried out, the two youngest (R2 and R7) died of wound infection about 2 weeks after operation. At the site of the trephine opening there was a thick layer of pus and no traces of osseous regeneration. Of the 3 surviving rabbits, one (R16) had been operated on at the age of 3 weeks, and in this animal regeneration had occurred at the margins of the defect and there were also some isolated bone islands, but 3 months after operation the centre of the defect was still unossified over a large area consisting of dense connective tissue only (fig. 8 A, a). In the second rabbit (R28), on which trephination had been performed at the age of 1½ months, regeneration was comparatively good; after 3 months there was only



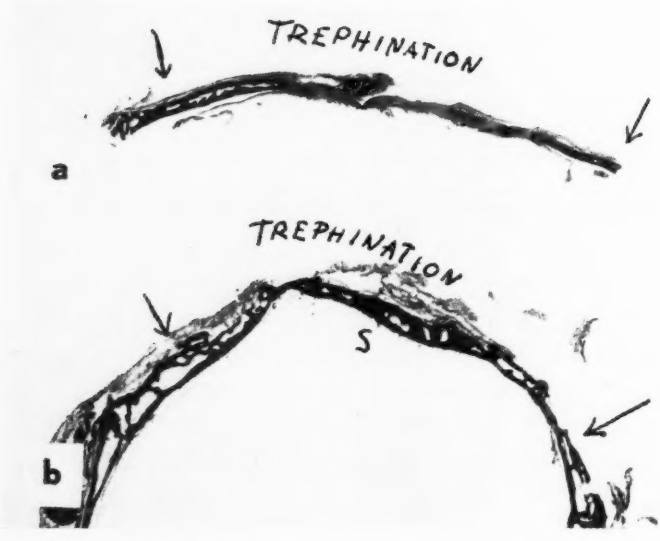


Fig. 8. — Calvariectomy (see fig. 3). *A*. A rabbit operated on at the age of 3 weeks (R 16). Radiogram of the calvaria 3 months after operation. New bone mostly at the margins of the opening. Note separate bone islands on the dural surface in the centre. *B*. Radiogram 5 months after operation on the calvaria of a rabbit (R 41) operated on at the age of 9 weeks. The opening has ossified but its centre consists of numerous separate bony plaques. *a* and *b*. The corresponding histological specimens.

at the site of the sagittal suture an unossified fissure 1 to 2 mm wide and in some places some small ossification defects. In the third rabbit (R41), operated on at the age of 9 weeks, the calvarium had regenerated within 5 months, although the top of the cranial vault consisted of separate bone plates joined together (fig. 8 B, b).

In addition to the previously mentioned animals, 2 more of the 56 rabbits of this group died. One of them (R 18), a rabbit operated on at the age of 1 month, died 2 months after operation in some kind of convulsions. The other, an adult rabbit (R 46), died 2 weeks after operation, probably from diarrhoea.

GROUP B

In this group, which comprised 6 young rabbits (6 to 8 weeks old) a similar trephination was performed, but the pericranium was freed as a wide lateral flap, replaced after the bone had been removed, and fixed with some silk sutures to the contralateral half of the calvaria.

Regeneration took place in more or less the same way as in the corresponding age group in group A. However, 4 months after trephination the structure of the new bone was both radiographically and histologically almost normal and it thus seemed that the outer periosteum had somewhat promoted regeneration (fig. 9). Yet here, as in the former group, there were additional sutures in the regenerated region. Such a suture was also formed at approximately the site of the coronary suture. One animal (R 57) had an intracranial haematoma, and, its condition being poor, was killed 1 month after operation. The calvaria was comparatively well regenerated.

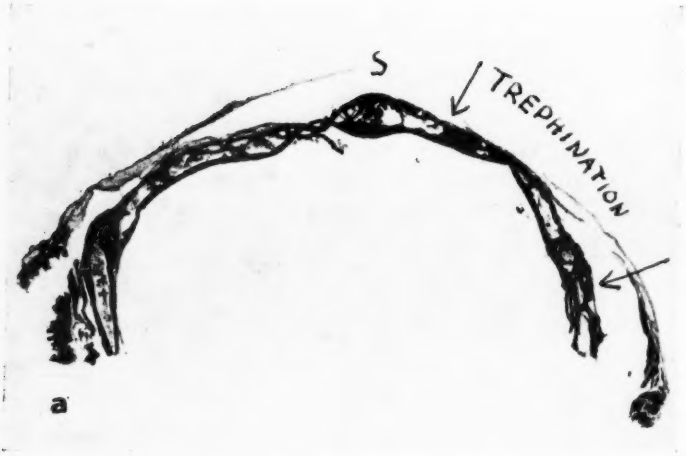


Fig. 9. — A case (R 62, Group B) in which standard trephination was carried out on the right side 4 months earlier, the pericranium being only temporarily freed as a lateral flap and replaced in its original site. The age of the rabbit at operation was 1 ½ months. It is difficult to see from the radiogram which side is the trephined one. In the regenerated bone the coronary suture is bifurcate. *A*. Radiogram. *a*. Histological specimen.

GROUP C

In this group, which comprised 12 rabbits of varying age, trephination was carried out on the right fronto-parietal region, the outer periosteum being removed from the operative area (standard trephination). Thereafter a thin polythene film (Polystan film, thickness 0.05 mm, Messrs. A/S Ferrosan Medicinalfabrik, Copenhagen), slightly larger than the bone defect, was placed on the dura mater, the edges of the film reaching a little below the bone margins

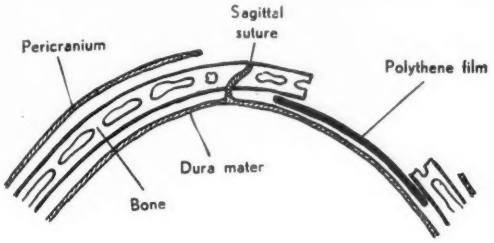


Fig. 10. — How the polythene film was placed in the trephine opening over the dura (Group C).

(fig. 10). In 2 cases the outer periosteum was also preserved and replaced over the defect and the polythene film.

In these animals of varying age regeneration seemed to take place to approximately the same extent as in group A, despite the presence of the polythene film. A firm connective tissue capsule formed around the film. The bulk of the new bone was found to have grown between the dura and the polythene film. If the outer periosteum had been removed at operation in the usual way, hardly any bone had formed on the outer surface of the polythene film, there being on that surface an aperture formed by the bony edges and approximately corresponding to the original defect (fig. 11 a, a'). Microscopic examination of these bone edges showed a small amount of new bone formation (remodelling) but it was obvious that there was very little growth towards the centre of the defect. In one rabbit (R 63), however, which had been operated on at the age of 4 days and was examined 4½ months later, a very thin incomplete bone bridge had formed over the polythene film while on the side of the dura there was a strong layer of bone without any defects. In the 2 cases (R 73 and R 74) in which the outer periosteum had been preserved, there was considerably more

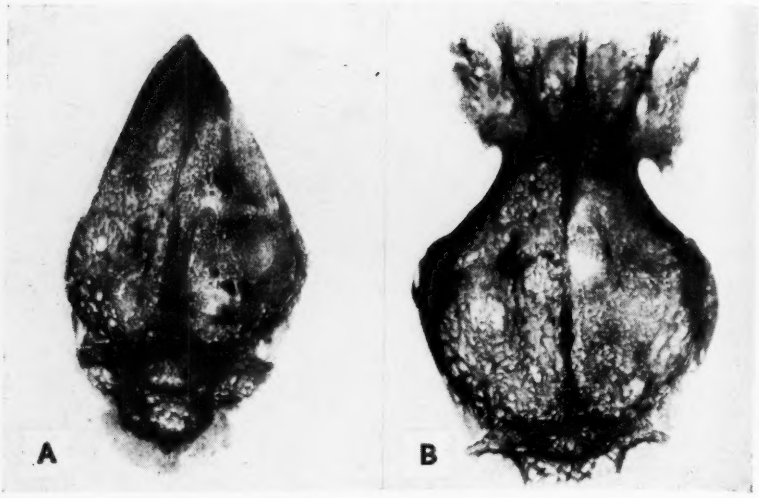


Fig. 11 A, B.

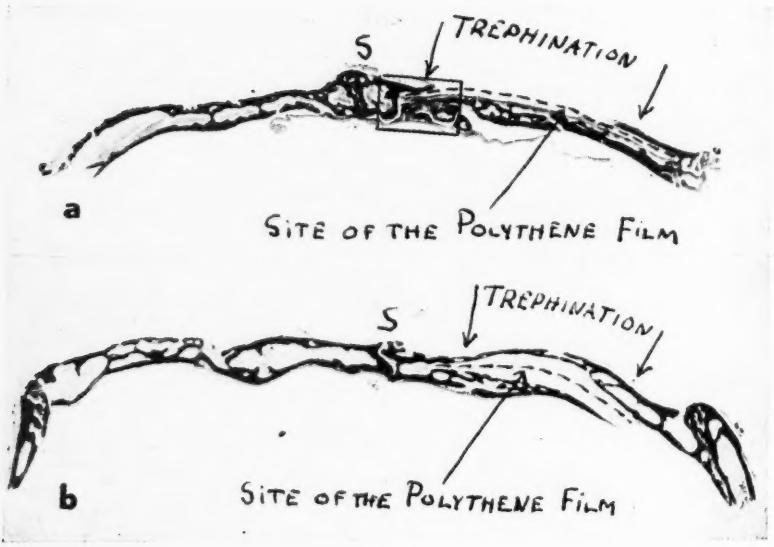


Fig. 11 a, b.

extensive, though very thin, bone formation on the outer surface of the film. This bone layer was thickest at the lateral margin where correspondingly the bone on the dural side was thinnest (fig. 11 b).

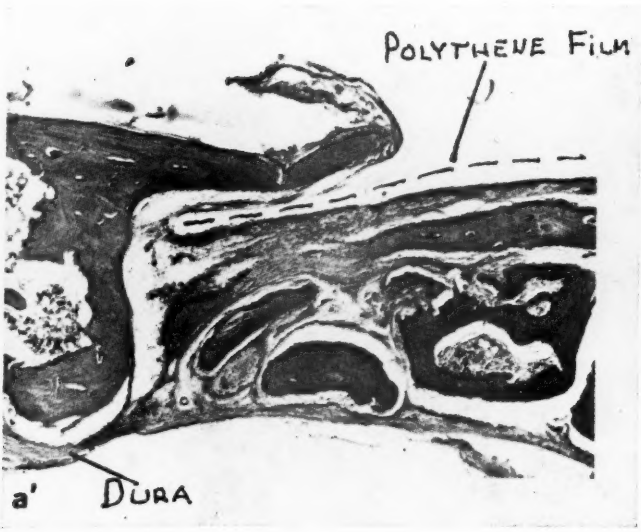


Fig. 11. — Examples from an experimental series (Group C) in which a polythene film was placed over the dura after trephination (see fig. 10). *A*. Radiogram of the calvaria, trephined when the rabbit (R 67) was 5 weeks old, 3 ½ months after the operation.On the trephined side the »coronary suture» makes a wide bend anteriorly. *a*. The corresponding histological specimen. The site of the polythene film and the thin bone margins above it can be seen. Between the polythene film and the dura a strong layer of new bone of fairly even thickness has formed. *a'*. Magnification of the former, the region of the medial bone margin. × 40. *B*. A rabbit (R 74) operated on at the age of 1 ½ months. In this case the temporarily freed pericranium was pulled over to cover the polythene film. Radiogram 6 months after operation. The structure of the calvaria is similar on both sides but for a missing coronary suture on the operated (right) side. Neither could this suture be found in a histological specimen from the normal site of the suture. *b*. The corresponding histological specimen. Medially the bone layer under the polythene film is thick, thinning gradually in a lateral direction. In the bone layer over the polythene film the condition is reversed.

Radiograms showed that in the operated area the entire coronary suture or the greater part of it had disappeared in all but one case (R 67, fig. 11 A). Here in the corresponding site there was a suture which, however, made a sharp bend and ran for about 8 mm in an anterior direction (possibly around the polythene film). In specimens taken from the site corresponding to that of the coronary suture no suture whatsoever was microscopically demonstrable in 6 cases. In 2 cases (R 64 and R 71) in which ossification of the trephine opening was only partial, there were between the bony plates two thin connective tissue strips of the type that is generally found in regenerated areas of the calvaria.

GROUP D

In this group, consisting of 4 young rabbits, standard trephination was carried out on 3 and calvariectomy on 1 animal. The defect was isolated from the surrounding bone either partially or completely by fixing polythene strips over the edges with some silk sutures (according to Ingraham).

In the two cases (R 77 and R 78) in which the bone edges were isolated all round the defect, bone had formed in the shape of islands on the surface of the dura. However, the defect was incompletely covered. In the rabbit (R 78, fig. 12 B, b, b'), in which a calvariectomy had been carried out, thin bone islands were only formed near the edges of the opening. The middle of the opening was filled with dense connective tissue which comprised the dura. In the second case (R 77, fig. 12 A, a, a'), in which a triangular opening 5 mm wide had been made in the right fronto-parietal region, ossification was better: the opening was largely covered by a bridge of bone plaques separated by connective tissue and thinner than the surrounding bone. The margins of the defect had become rounded in the shape of the polythene strips and were sharply delineated from the surroundings in the radiograms. In another 2 cases (R 75 and R 76) in which only part of the surrounding bone margins was isolated, ossification was good within 3 months except in the immediate vicinity of the film strips.

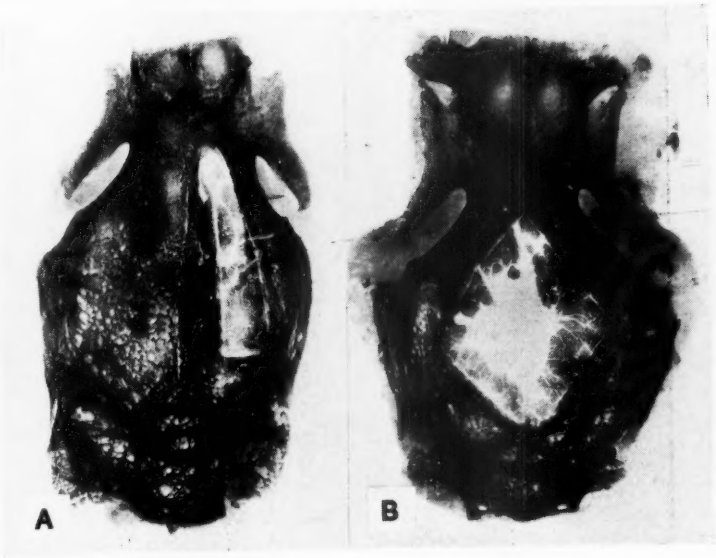


Fig. 12 A, B.

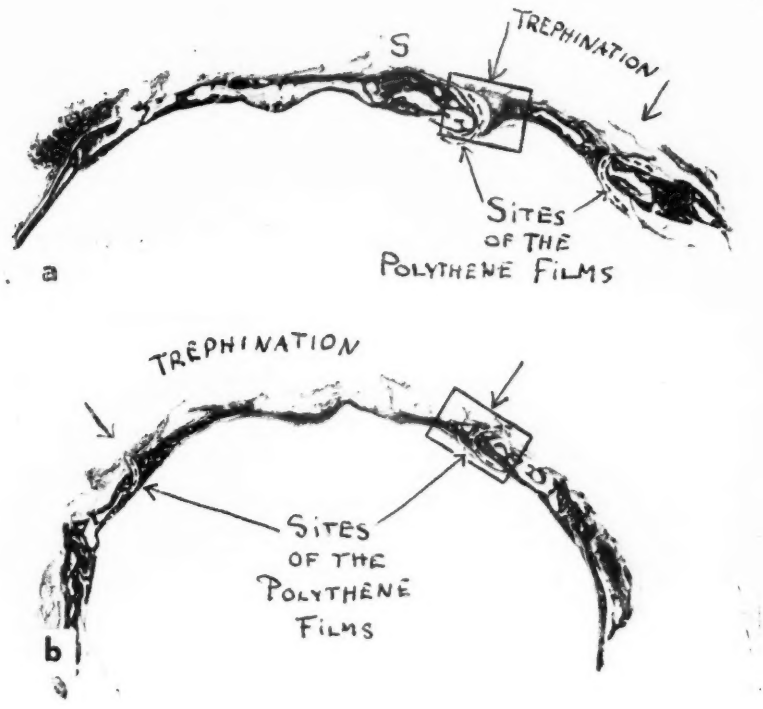


Fig. 12 a, b.

Fig. 12. — Cases in which the margins of the trephined opening were lined with polythene strips (Group D). In both the above cases (R 77 and R 78) the age at operation was 1 ½ months and the postoperative observation time 3 months. In radiograms *A* and *B* the margins of the trephine opening can be clearly distinguished and also the bone islands in the opening, which are thinner than the surrounding bone. Note the ossification defect in the centre of the latter picture. In the histological specimens *a* and *b* we find that the margins of the defect are rounded under the polythene strips and that the new bone penetrates in the shape of a thin trabecula between the polythene strip and the dura. *a'* and *b'* are the respective magnifications (× 40) of the areas outlined at the margin of the defect. Note a thin bridge of new bone around the edge of the polythene strip on the dural side in the latter picture.

GROUP E

In 5 rabbits the dura mater was removed from the area of the bone defect in connection with standard trephination. Further the defect was in one case covered with pericranium which had been pulled aside for the duration of the trephination (as in group B). In another 2 cases the defect in the dura was covered with polythene film over which the pericranium was drawn.

In all these cases the result 2 or 3 months later was a bone defect which roughly corresponded in size with the original trephination defect. The margins, however, had become rounded. Macroscopically the defect looked quite different from those in the former experiments: it was covered by a clear transparent connective tissue

Fig. 13. — The dura mater has been removed from the area of the defect (Group E). In the trephine opening in a young rabbit (R 79) normal regeneration has not taken place and the opening is covered by transparent connective tissue. *A*. In the radiogram the defect is well-defined. Whether the small indistinct shadows in the defect are caused by minute bone splinters persisting after the operation or whether there is some insignificant bone formation at these points, cannot be determined. *a*. Histological specimen. *a'*. Magnification of the square area outlined on it (× 40). The bone margins are rounded and the dura covers them, changing at the outer surface into pericranium. In the defect there is loose connective tissue and as a sign of close cerebral attachment a considerable piece of brain cortex.

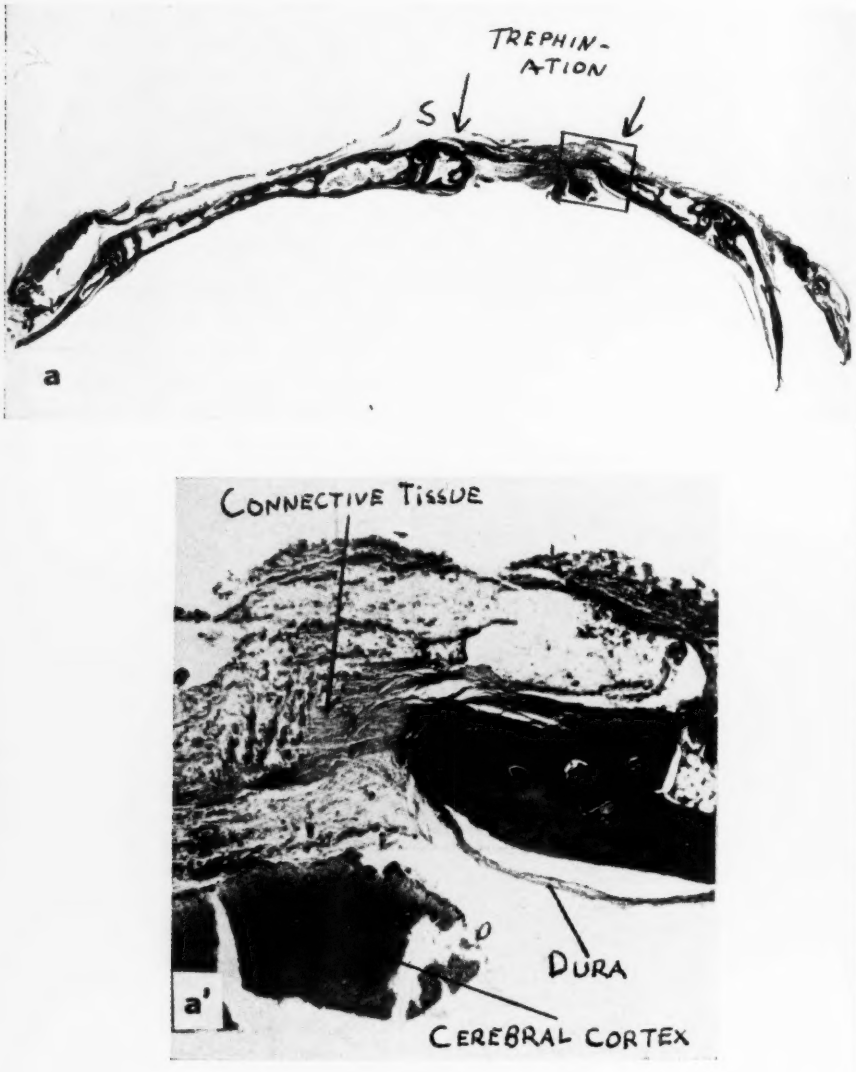


Fig. 13 a, a'.

membrane. Microscopic examination showed the bone margins to be thick and rounded in the same way as in the former group when isolated with polythene strips, and that the edges were covered by a layer of fibrous connective tissue fusing with the pericranium on the outer surface and with the dura on the inner (Fig. 13 a'). In the opening there was rather loose connective tissue

tightly adhering to the brain cortex if this had been left exposed (R 79 and R 83, fig. 13). Such tight adhesion did not occur in those cases (R 80, R 81 and R 82) in which the dural defect had been covered by polythene film or by pericranium; there were only minor adhesions at the margins of the opening. In each of the 3 last-mentioned cases one solitary well-developed bone island (Fig. 14) was observed which was connected with the marginal bone by a narrow bridge and which quite obviously originated from the dural side (it was situated under the polythene film in cases R 81 and R 82). Through this isthmus ran a macroscopically demonstrable

Fig. 14. — In a young rabbit (R 81) the dura mater was removed from the trephine opening, and the dural defect covered with a polythene film over which the pericranial flap was drawn. After 3 months the opening is completely unossified with the exception of a solitary bone island connected by an isthmus with the posteromedial margin of the defect and situated under the polythene film.

connecting blood vessel. One explanation of the occurrence of this kind of solitary bony formation would seem to be that when the dura was removed at operation some dural vessel might have escaped division and have been left *in situ* with a small dural strip at the site of the defect covered by the polythene film or by the periostal flap. These bony islands did not join the margins at any specific point.

GROUP F

This group comprised 8 cats of varying age. The usual trephination was carried out on the right fronto-parietal region, or on the parietal region alone.

The cats were killed 4 months after operation with the exception of one adult cat (C 8) which was killed after 5 months. When the regeneration of the defect in these different individuals was studied on the basis of radiograms (fig. 15), the same observation was made as with regard to rabbits of different ages: the younger the animal, the more obscure were the margins of the defect (more rapid remodelling). Closure of the defect in the cats was somewhat different from that in rabbits. In a kitten operated on at the age of 6 days (C 1) the defect had ossified completely but the new bone layer was slightly thinner than normal. In 3 kittens operated on at the age of 17 days (C 2, C 3 and C 4) ossification was almost complete; in each of these cases there remained a small defect, however. In a kitten operated on at the age of 1 ½ months (C 5) the defect was incompletely covered by bony protuberances and islands. In all adult cats (C 6, C 7 and C 8) a thin layer of new bone occurred chiefly at the margins of the opening, while considerable ossification defects persisted in the centre.

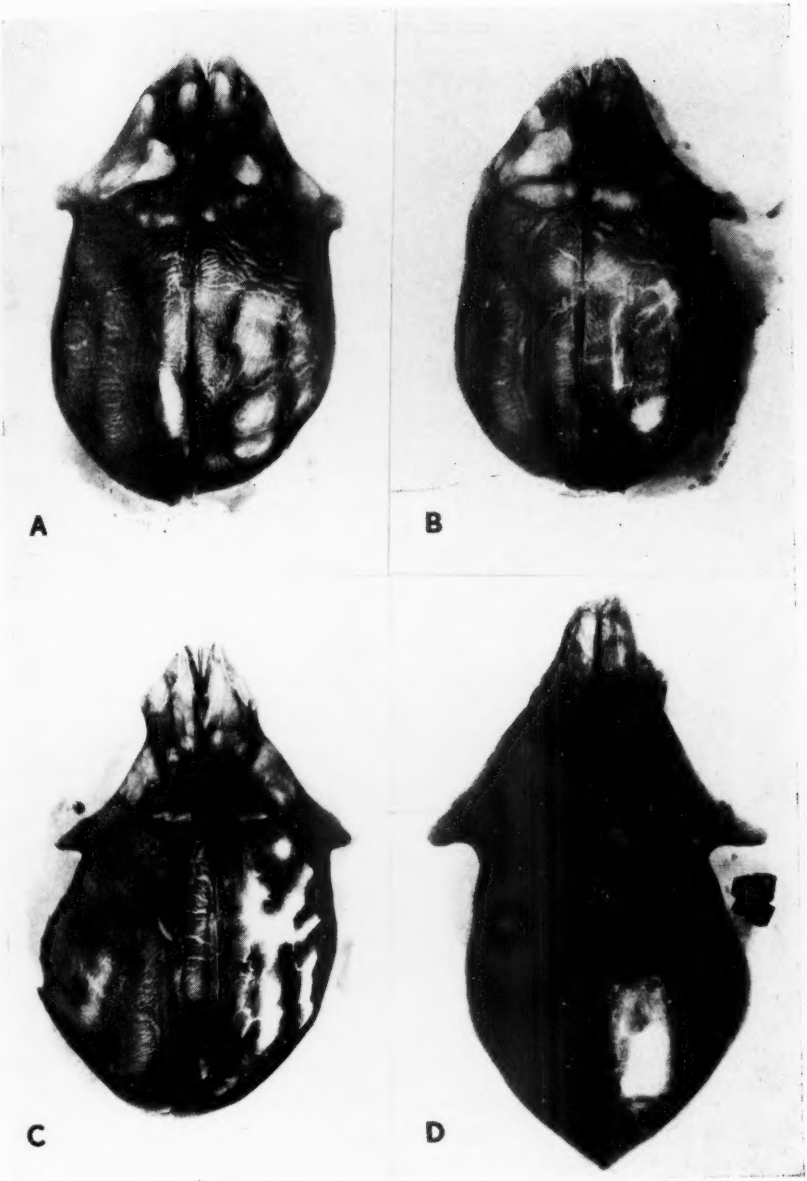


Fig. 15. — Radiograms of the calvariae of 4 cats 4 months after trephination (Group F). The age of the animals at operation was as follows: *A*, 6 days old (C 1), *B*, 17 days old (C 4), *C*, 1 ½ months old (C 5) and *D*, adult (C 7). Note that in the adult cat the contours of the rectangular trephine opening can still be seen.

DISCUSSION

SIMILARITIES AND DIFFERENCES BETWEEN THE CALVARIAE OF MAN AND OTHER MAMMALS

The calvaria, or cranial vault is best defined as the portion of the skull above a plane drawn through the supra-orbital ridges and the external occipital protuberance [26]. Thus it consists of the following bones: the parietal bones, the squamous portion of the occipital and temporal bones, the squama frontalis, and a small portion of the great wing of the sphenoid. In exceptional cases the human calvaria may also comprise the interparietal or Inca bone. This bone is regularly present in some mammals, *e.g.* rabbits [20, 51]. According to another definition, the calvaria is slightly narrower, its lateral border following the temporal line [132].

Despite certain differences, the embryonic development of the calvarial bones of different species of mammal, their microscopic and macroscopic structure and their function are in principle the same [20, 22, 112, 140]. These bones are membrane bones, *i.e.* there is no cartilaginous transitional stage in their development.

For details of the embryogenesis and structure of the human calvaria and sutures, reference can be made to various textbooks of anatomy and histology [60, 108, 132], and to many other sources [10, 41, 88, 89, 129, 148]. Bruce [20], who particularly investigated the embryological development of the skull of the rabbit, stated that the order of occurrence of the ossification centres is largely the same in man and other mammals.

The Embryological Development of the Calvaria of the Rabbit According to Bruce. — In the rabbit, as in other mammals, the bones of the calvaria are formed in the connective tissue membrane covering the brain, and bone formation proceeds from separate points known as ossification

centres. In the beginning these centres consist of fine reticular bone tissue or of ossified spicules. The first of them to appear in the foetal development on the seventeenth day, are the parietal ossification centres, one on either side. The second are the ossification centres of the frontal bone appearing on each side on the nineteenth day. The squamosal bone of the temporal complex occurs on the twentieth foetal day in the shape of two separate ossification centres, which soon fuse. The occipital bone, or »occipital complex», of the rabbit consists of four bones: the supraoccipital, two exoccipitals and the basioccipital bone; in the centre of these there is the occipital foramen. The supraoccipital bone which, unlike the other three, originates in connective tissue, adjoins the posterior part of the calvaria. Its two ossification centres appear on the twenty-first foetal day and fuse within one or two days. The interparietal bone appears between the parietal and supraoccipital bones from either one or two ossification centres between the twenty-first and twenty-third days. The growing bones of the calvaria radiate out from the ossification centres and by the end of the gestation period of thirty days the bones are separated only by sutures and fontanelles. Differences in the pattern of ossification were not observed in different rabbit breeds.

The adoption of an upright posture, the use of the upper extremities for procuring food and for defense, and the consequently reduced importance of the jaws have probably been of significance in the phylogenetic development of the human skull. During the course of this development the brain and the cerebral cranium have increased in size at the cost of the visceral cranium. The fact remains that, corresponding primarily to the large size of the pallial portion of the brain, the human calvaria is proportionally much larger than that of any other mammal. During the course of phylogenetic development the area of the cranial surface which is covered by muscle (temporal muscles and muscles of the neck) has proportionally diminished in size. The human calvaria is covered by a fibrous sheet, the galea aponeurotica, which corresponds to the platysma of the neck [3]. The mechanical traction of muscles may perhaps influence the shape and ossification of the calvaria [160, 165].

The bones of the human calvaria have no nutrient artery such as the other bones of the skeleton usually have [3]. According to the investigations of Hyrtl [73], Langer [91] and Goldhahn [51], these bones receive their blood supply from arterioles principally emanating from the dural arteries to the inner surface (the most important of these being the middle meningeal artery) and to a lesser extent from the small arterioles of the outer surface, the

majority of the latter emanating from the superficial temporal artery [141]. The largest artery penetrating the calvaria is about 1 mm in diameter, running on either side through the parietal foramen. This artery, however, is an anastomosis in character. Most of the blood inside the bone is venous blood which slowly moves along the diploic veins [3]. At the site of the muscle insertions there are more blood vessels than elsewhere in the bone [51]. Practical experience in the field of neurosurgery has taught that a substantial amount of arterial blood enters the calvaria through the insertion of the temporal muscle [90]. It is possible that due to the comparatively large size of the calvaria and the comparatively small number of muscle insertions the circulation of the blood is poorer in the human calvaria than in that of other mammals. The general regenerative capacity may also differ in different species of mammals.

ON THE RATE OF HEALING OF DIFFERENT BONES

The time necessary for the healing of the various bones of the human skeleton differs, depending on various factors. We know that fractures of the lower third of the diaphysis of the tibia, humerus and ulna and certain fractures of the carpal scaphoid bone tend to heal slowly, while, on the other hand, fractures of the metaphyses heal comparatively quickly. One explanation of slow consolidation is that when a bone is fractured a highly important intra-osseous arterial connection of one of the fragments is broken, resulting in a greatly reduced blood supply. Regeneration may also be delayed by such factors as wide separation of the fracture ends, interposition of adjacent soft parts and infection. The healing time of long bones further depends on whether the fracture is oblique or transverse and on the patient's age. Further, individual variations occur to some extent [161].

Our knowledge of the regenerative phenomena of bone is largely based on those numerous investigations that have been made on the healing of long bones. The following facts place the bones of the calvaria in a different category from the other bones of the skeleton: 1. The bone in question is a large flat structure which is covered by periosteum on either side: the pericranium and the dura mater. 2. The function of the calvaria is primarily that of protection, and

the bearing of the mechanical stress exerted by muscles is only a secondary function. 3. In the absence of muscular traction there is neither an approximation nor a pulling apart of the fracture surfaces. Neither do the bone fragments usually move in relation to one another in fractures of the calvaria. 4. The bones of the calvaria have no nutrient arteries but their nutrition is dependent on small separate arterial branches to the bony surface. 5. In connection with fractures or defects in the bone, brain substance may protrude into the defect (cerebral hernia) if the dura has been injured.

REGENERATIVE CAPACITY OF THE CALVARIAL BONES

The prevalent opinion is that regeneration in openings made into the adult human calvaria is insignificant so that these defects remain patent, although contradictory opinions have also been presented in connection both with findings of Peruvian skulls [136, 153] and with some clinical cases [9, 53, 104]. On the other hand, we have good reason to believe that openings trephined in the skulls of infants ossify entirely [49]. Animal experiments have shown that the regenerative capacity of the calvaria of young rabbits, guinea-pigs and dogs [157] and young rats [147, 155, 159] is strikingly good, although observations to the contrary have been reported [12]. On the other hand, it has been claimed that defects in the calvaria of adult guinea-pigs [157] and adult rats and dogs [47] do not heal. In my experiments good regeneration, complete bony closure of the defect, was observed, particularly in young rabbits and cats. A considerable degree of regeneration was also found, however, in adult animals. Thus it seems obvious that the regenerative capacity of the calvaria in experimental animals is better than in man.

THE COURSE OF REGENERATION

In investigations of a given histological phenomenon, conclusions generally have to be based on observations on a series of tissue specimens representing different stages of the process in different individuals. The forming of an opinion on a kinetic occurrence in this way always necessitates certain assumptions. From a study of

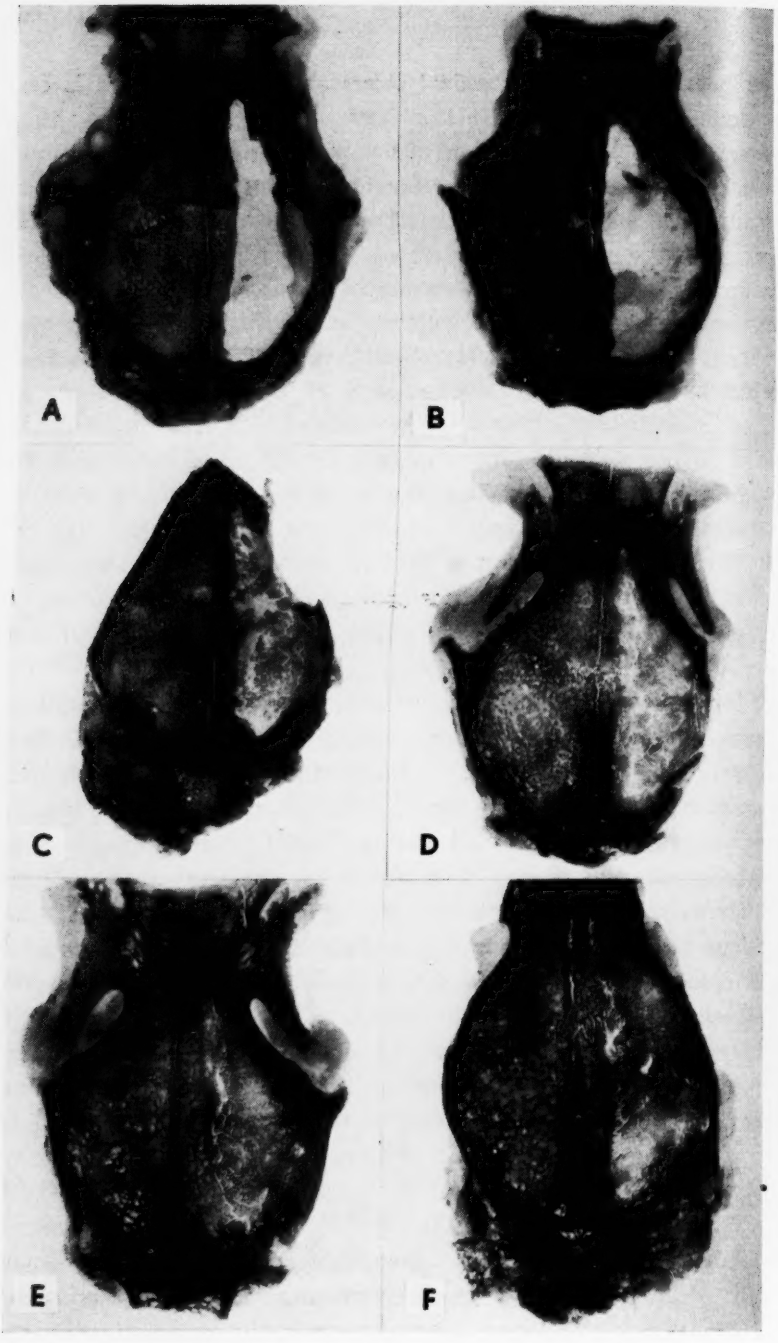


Fig. 16. — The course of regeneration of the calvaria of a young (6—8 week old) rabbit demonstrated by radiograms. The postoperative periods are *A*, 3 days (R 34), *B*, 8 days (R 29), *C*, 2weeks (R 22), *D*, 1 month (R 23), *E*, 2 months (R 36), and *F*, 4 months (R 33). The first signs of the formation of new finely granulated bone are radiologically demonstrable after 8 days (*B*). The contours of the trephine opening disappeared from the radiograms within 2 months (*E*). Note the presence of secondary sutures.

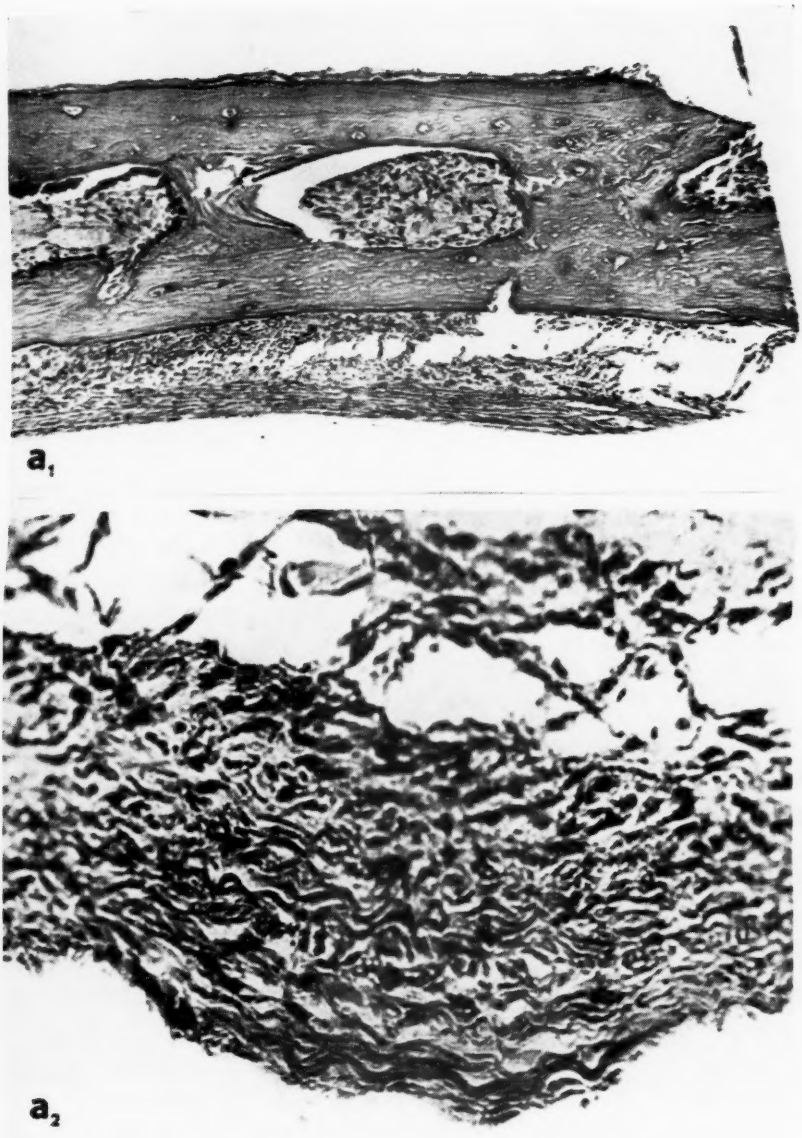


Fig. 17. — a_1. The medial margin of the trephine opening 3 days after operation (R 34). A zone of the margin is dead; the nuclei of the osteocytes have disappeared from this zone. Note also the disappearance of the row of osteoblasts lining the diploic space in the dead zone. The pericranium is excised from the outer surface of the bone edge. The osteogenic cell layer between the dura and the bone is markedly proliferated. The inner fibrous layer of the dura is swollen and its fibers somewhat scattered, while at the same time the dura as a whole has drawn away from the bone. × 100. a_2. Similar changes are observed in the dura in the centre of the defect under the haematoma. × 400.

my own preparations I have obtained the following idea of the course of regeneration of the calvaria of a 6-to 8-week-old rabbit.

The trephine opening is first filled by a haematoma. *Three days after operation* (fig. 17) a narrow strip of the bone edge has undergone necrosis. This is evidenced by the fact that the nuclei have disappeared from the lacunae of the osteocytes [15, 61]. Therefore the expression »dead bone» has been used in the present paper to mean bone matrix without any nuclei. When the pericranium at the medial margin of the opening was resected farther (to as far as the other side of the sagittal suture), the dead bone is generally found to extend farther in the outer table than in the inner table. Dead bone is also sometimes found on the surface of the opposite parietal bone in the region from which the pericranium has been removed. In the region of the opening and for some distance under the bone margins the dura mater is considerably thickened. Under the bone edges the osteogenic cells of the dura have proliferated considerably. A similar cell layer, varying somewhat in thickness, is seen on the dural surface under the haematoma. These cells are here called osteogenic cells and it seems that they might be distinguished from ordinary connective tissue cells, not only by their site, but also by the fact that they are more closely grouped together. Furthermore, the layer of dense connective tissue of the dura has somehow become swollen and sparser. Proliferation of a similar kind is also observed under the pericranium at the site where it has been devided. *Five days after operation* (fig. 18) the cell proliferation of the dura has proceeded further. The »granulation layer» merging into the dura under the haematoma has become markedly thicker, forming a bridge between the bony margins. On the outer surface there is connective tissue which seems to invade the haematoma. Attached to the outer surface of the bone at the site from which the periosteum has been removed a thick layer of connective tissue is observed. Already at this point some trabeculae of new bone of the very earliest stage can be seen within the mentioned granulation cell layer (callus plate), which merges the dura. Around such a typical primitive bone island there are typical active osteoblasts [128], the nuclei of which are large, angular and deeply stained. *Eight days after operation* the haematoma has been partly resorbed. In the callus layer there begins to appear more bone trabeculae at slightly varying levels.

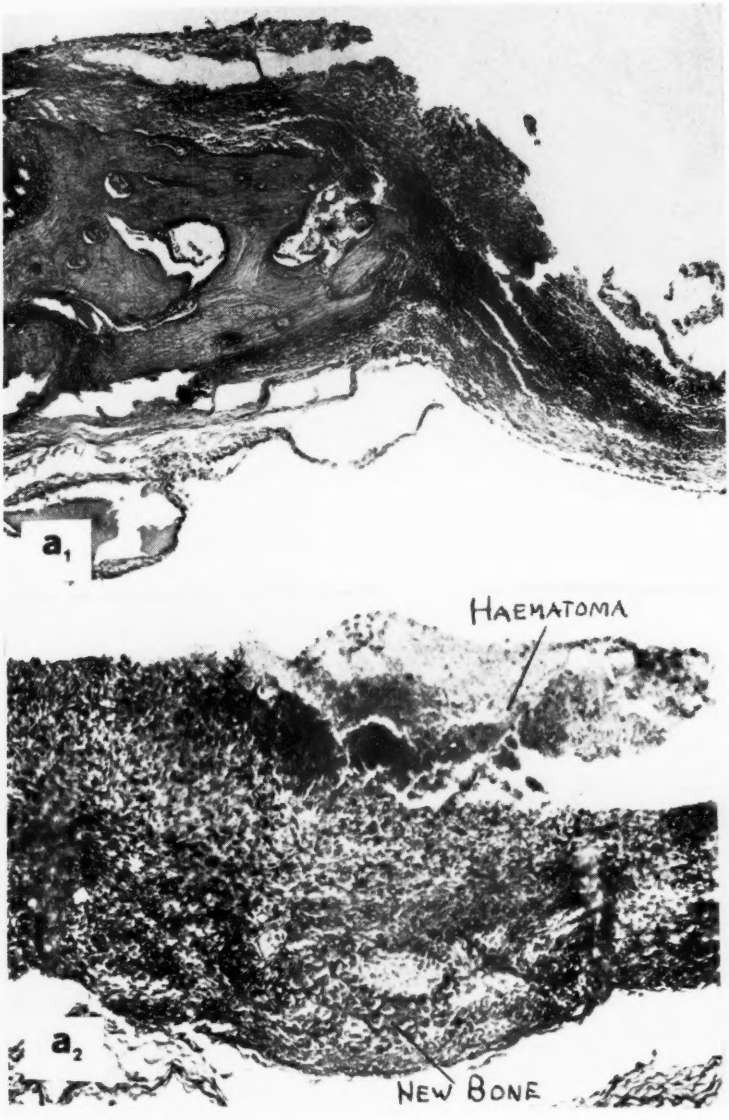


Fig. 18. — The medial margin of the trephine opening 5 days after operation (R 35). The callus plate is forming. a_1. The dural cells show marked proliferation. Of the haematoma on the dura (right) only remnants can be traced in the picture of the specimen. On the outer surface of the bone there is also loose tissue resembling granulation tissue. × 60. a_2. The dural cell tissue is markedly proliferated (note that the magnification is one-fourth of that in *fig. 17* a_2). On the developing callus plate part of the haematoma is seen. Active osteoblasts with deeply staining nuclei begin to appear in places and in connection with them here and there primitive bone trabeculae. × 100.

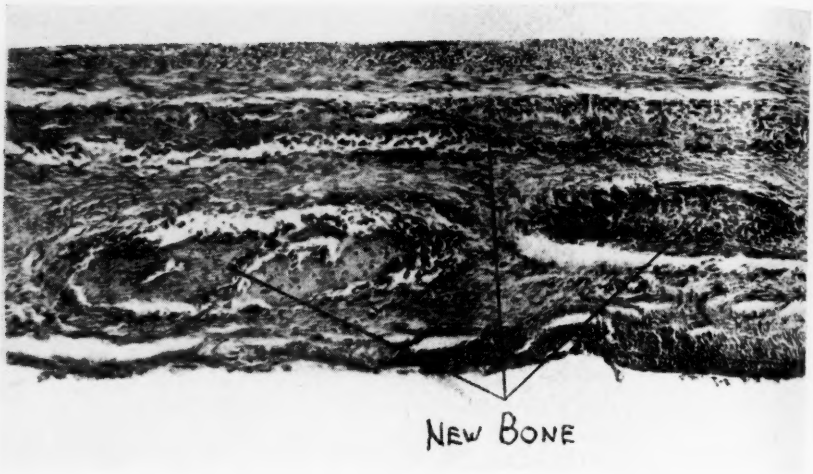


Fig. 19. — The callus plate 2 weeks after operation (R 22). The osteogenic layer is now well-defined, forming a bridge between the bone margins. Islands of primitive bone surrounded by thick layers of osteoblasts can be seen at various levels of this plate. The connective tissue begins to differentiate on both the inner and the outer surface into the dura and the pericranium respectively. × 100.

From now on the radiograms (fig. 16 B) begin to show fine bone granules at the site of the defect. Their grouping is somewhat irregular and they seem to occur mostly at the lateral parts of the opening. *After two weeks* the osteogenic layer between the bone margins can readily be distinguished microscopically because there has arisen a comparatively thick layer of irregular bone islands and trabeculae of varying size (fig. 19). Bone formation is more marked at the margins than in the centre of the defect. It seems as if the islands had in places fused to form a continuous system of trabeculae. Large numbers of osteoblasts can be seen around the primitive bone. The outer connective tissue seems in places to join the callus layer without any detectable limit. The dead bone at the bone margins has obviously been resorbed to some small extent and most of the remaining portion has been covered by new bone. Thus a thin new bone layer has formed on both the dural and the pericranial surface here, and at the margins new bone has grown which protrudes into the callus layer in the shape of tortuous trabeculae. In the old dead bone matrix some blood vessel tunnels can be seen with new bone in their walls (fig. 20). In the

Fig. 20. — Remodelling of the bone edges. Two weeks after trephination dead bone partly surrounded by new bone in the margins of the defect can be seen in a young rabbit (R 22) as shown in the picture above left. Below in this picture there is a blood vessel surrounded by cells that appear to be osteoblasts. From the blood vessel a bud branches into the bone matrix above. Around the bud there are some new bone cells in the tunnel wall. Above right there is another larger tunnel. × 400.

radiogram (fig. 16 C) the bone granules are seen to have become denser, covering the opening fairly evenly; the margins of the trephine opening are still sharp. *A month after operation* (fig. 16 D) the new bone layer is already remarkably homogeneous, although it consists largely of bone plates separated by thin connective tissue walls. The bone is primitive but no longer trabecular in structure, and in places an outer and an inner table can be distinguished. Besides the narrow »sutures», there are here and there wider connective tissue areas in which occur small primitive bone islands surrounded by thicker layers of osteoblasts. The dural connective tissue has become denser, being still decidedly thicker in the regen-

eration area than on the opposite side of the calvaria. A fibrotic layer corresponding to the pericranium has formed on the outer surface of the bone. On both surfaces of the bone there is a regular row of osteoblasts. Dead bone surrounded by new bone can still be observed at the margins of the defect. In the radiogram the edges of the defect have become blurred and, besides a continuous bone layer, which seems somewhat thinner than its surroundings, several intersecting connective tissue sutures can be seen traversing the opening. *After two months* (fig. 16 E), bone organization has proceeded still further. A large part of the bone is lamellar although primitive bone areas can also be observed, particularly near the extra sutures. Diploic spaces and normal bone marrow can be seen in places, generally in the lateral parts. The thickness of the new bone layer is radiologically nearly normal. *During the next two to three months* the bone changes comparatively little. The organization of the bone tissue improves during this period and the thickness of the fibrotic layer that corresponds to the dura and the pericranium becomes almost normal. Radiologically (fig. 16 F) the two halves of the calvaria begin to look more and more alike. Besides suture formations in various directions, the coronary suture, though less tortuous than normal, occurs fairly constantly in the regeneration area.

From this time on, the development of the regenerated bone seems gradually to conform with the general growth process of the bones of the calvaria. It seems obvious that from this point onwards growth in thickness takes place in the normal way by apposition to the flat surfaces of the bone. Growth in width, however, may take place both at the sites of normal sutures and in the same way at the extra sutures also, judging from the signs of bone growth at these sites.

It should be noticed that the first phases of regeneration described above rather resemble the embryogenesis [122, 152] of the bones of the calvaria. The callus plate here corresponds to the embryonic membrane which »changes» into bone. However, in the course of regeneration of the calvaria I have not been able to see any correlation between the formation of new bone and the original ossification centres.

ON WHAT LOCAL FACTORS DOES REGENERATION OF THE CALVARIA DEPEND

Two theories have been advanced to explain the formation of new bone. According to the first of these, the theory of specific osteogenic cells [39, 43, 44, 52, 86, 98, 116, 117, 128, 133] the osteoblasts are the primary bone cells which possess the capacity to form new bone. Against this theory another, the theory of metaplastic bone formation, has been presented [2, 5, 11, 13, 66, 67, 72, 95, 96, 97, 118, 162] according to which the osteoblasts only represent an intermediate morphological stage, arising by way of induction from connective tissue cells, apparently from undifferentiated mesenchymal cells, and developing further into mature bone cells (osteocytes). It has further been asserted that new bone arises from the endothelial cells of the capillaries [33, 83, 115], or from perivascular reticular cells [68]. As to healing of a fracture, opinion is fundamentally divided with regard to whether the bone-forming cells of the callus originate from the fractured bone and its periosteum, *i.e.* are proliferating osteoblasts, or from primitive cells of the outer connective tissue. It can be established that on either side of the fracture, and under the periosteum in particular, the osteogenic cells divide mitotically and multiply abundantly [61]. On the other hand, it is difficult to distinguish between osteogenic cells and ordinary connective tissue cells before they begin to form bone. Primitive bone occurs as trabeculae in the callus mass [6, 61, 164].

It has been suggested that the bones of the calvaria cannot form callus at all [62]. With very few exceptions [113, 126] cartilage, for instance, has not been found in connection with these bones [85, 155]. Yet Weinmann and Sicher [164] stress that the nature of bone is the same all through the skeleton, irrespective of whether it arises from cartilage or connective tissue. It may be considered a fact that the bones of the calvaria also form callus, although to a very small extent [61, 164]. A condition requisite for the development of cartilage is local stress [50, 81, 84, 142, 164], such as is present in fractures of the long bones. After trephination of the skulls of rabbits, a comparatively thick granulation layer, which seemed to merge into the dura mater (fig. 18), replaced the removed bone within a few days. Since new bone developed in this tissue plate in the shape of islands and trabeculae (fig. 19), there seems good reason to call it callus.

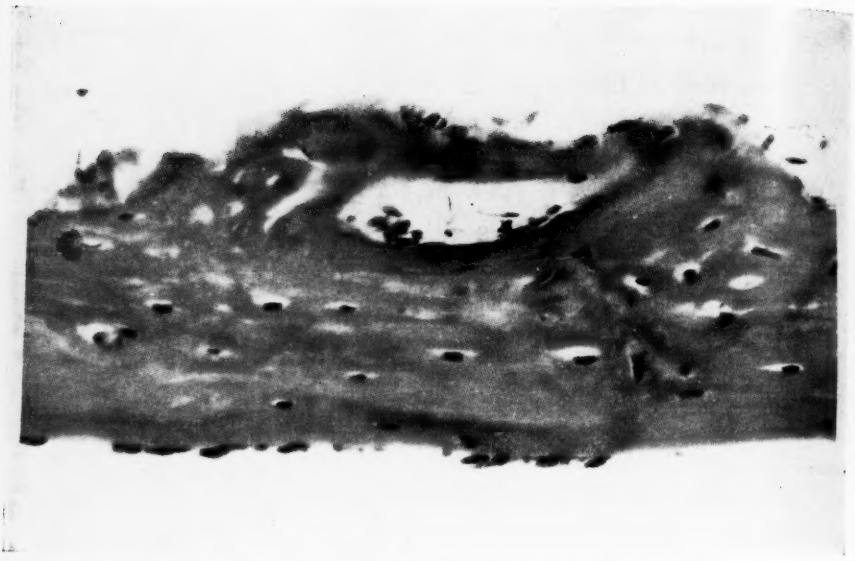


Fig. 21. — A piece of the calvarian bone removed at trephination from a 1½-month-old rabbit (R 28). On the inner surface of the bone an incomplete row of osteoblasts can be seen. × 400.

Some investigators [100, 147, 157] have laid great stress on the bone-forming capacity of the dura. Simpson *et al.* [147] pointed out that after trephination of young rats, bone in the shape of islands may occur on the dural surface. The reason for this they believed to be that at trephination part of the osteoblast layer between the dura and the bone remained on the dural surface and part on the bone surface (cf. fig. 21). According to their hypothesis, these cells would be the ones chiefly responsible for regeneration. A similar row of osteoblasts covers the bone both on the outer surface and along the sutures, as well as in the diploic spaces. It should be observed that in the histological specimens this cell layer seemed to be more or less loose, belonging organically neither to the bone nor to the membrane covering it (fig. 24); only at the sutures was it firmly fixed to the bone. This does not necessarily mean that conditions would be exactly the same in the living organism. When a polythene film was placed on the exposed dura at trephination, a thick layer of bone formed under the film (fig. 11 a). The fact that the lateral portion of the bone layer under the film was as a rule thinnest might be attributed to the mode of growth of

the calvaria. The decrease of the convexity of the calvaria as the brain grows would in this case occur as a result of resorption or delayed apposition at the inner surface of the bone in the lateral parts and increased apposition at the corresponding outer surface.

In man an extradural haematoma may occasionally become encapsulated in the bone [134, 149], as may also a dural endothelioma [166]. This may be considered an example of the bone-forming capacity of the human dura. The formation of such a capsule may take years [134]. In connection with atrophy of the frontal lobes in old persons, a corresponding thickening of the bone at the inner surface may take place while the outer surface withdraws but little [41]. This may be taken as proof of the capacity of the dura for slow bone formation even in old age. Erdheim [41] considered the firmer adhesion of the dura to the bone in old persons to be a sign of increased active bone formation, of about the same kind as in children.

In its relation to the bone the outer periosteum, or pericranium may well be compared with the inner periosteum, or dura mater [157]. As compared with the dura its importance in the regeneration of the calvaria is probably slight, however. In man the arterial branches entering the bone from the pericranium are fewer than those coming from the dura [51]. Moreover, when a trephine opening is made in the calvaria, the pericranium must be freed from its surroundings and possibly also from its vascular connections. On the basis of my own experiments it seems that a pericranium temporarily freed from the site of trephination preserves to some extent the capacity to form bone. When a polythene film had been placed on the exposed dura, a more complete, although thin, layer of bone was formed when the outer periosteum had been replaced over the defect than when the pericranium had been excised from the trephination area (fig. 11 b). The final ossification seemed, both radiologically and histologically, somewhat better if the outer periosteum was preserved (fig. 9). The pericranium must also be of importance for the nutrition of the outer table, since in some cases its removal resulted in death of the osteocytes at the outer bone surface. In the standard trephinations, particularly, the pericranium was excised more widely on the medial side of the opening. Whether this fact is responsible for the better bone formation in the lateral margin of the defect

cannot be decided. From the surrounding connective tissue a new pericranium developed at the site of the removed one.

Kochiyama [85] believed that ossification of openings in the calvaria of rabbits is only partial and that the principal agent governing the formation of bone is the marrow exposed at the margins. The changes that take place in the bone margins of the trephine opening have already been described in the foregoing. If the margins were covered with strips of polythene, bone islands were still formed in the defect (fig. 12). The bone edges became thick and rounded in these cases. However, if the calvaria was widely resected on both sides, bone formation was more abundant in the periphery of the defect (fig. 8). It is obvious that the bone edges, particularly the lateral edges, play their own part in the formation of new bone. For this the following possible reasons may be adduced: 1. The circulation is better in the area of the intact bone, particularly in its lateral parts. 2. In the periphery of the defect there are more osteogenic cells. 3. The bone margin offers better immobilization for the growing bone.

When bone is broken, the bone necrosis in the fracture ends and the resulting reorganization are obviously connected with the interruption of the blood supply in certain areas and the formation of new vascular connections. The fact that in my experiments the lateral parts of the calvaria seemed to regenerate better than the central parts, may be correlated with the fact that the arterial blood supply enters the calvaria from the sides. The bone-forming capacity of the dura mater must be seen in relation to the intact blood supply. In the cranial bones of young rabbits the densest vascular network is found at the sutures, that is in the area where bone formation is most vigorous. »Every bone regeneration process is intimately associated with an undamaged blood supply to the osteoblasts» (Rohde [133], 1925).

If the dura is removed from the trephine opening, the opening remains patent (fig. 13). This fact has been established by several previous workers [9, 100, 112, 147, 157]. In these cases the cerebral surface adheres very strongly to the outer connective tissue. This marked »interposition» of soft tissues may actually inhibit regeneration (cf. intrusion of muscle tissue between fracture ends in fractures of the long bones). Neither did the usual regeneration take place in those few cases in which, after excision of the dura,

the brain surface was protected from the outer connective tissue either with a polythene film or with replaced pericranium. In these latter cases, however, a solitary bony trabecula was growing from the margin of the opening (fig. 14); it might have originated from a piece of the dura attached to a blood vessel and left behind at the margin of the opening. In the absence of dura the pericranium was not observed to have formed bone.

After trephination, 2 of my experimental animals had a violent purulent wound infection which seemed entirely to prevent regeneration of the bone. It has been said that generally the healing of a fracture is delayed by simultaneous infection [161]. On the other hand, it has been claimed that bone infection may stimulate the regeneration of the calvaria [37].

In my experiments callus tissue, which seemed to merge without any limit into the thickened dura mater, developed at the site of the removed calvarial bone. The simplest explanation would seem to be that the most important of the factors responsible for callus formation is the rapid multiplication of the osteogenic cells remaining on the dural surface and also of similar cells in the diploic spaces and under the pericranium. In the subperiosteal osteoblast layer beside the fracture, an abundance of mitoses have been observed during the first few days of regeneration [61]. To some extent some of my own observations argue against the hypothesis that the callus originates from external connective tissue, possibly from the mesenchymal cells. In microscopic specimens from the immediate postoperative period the osteogenic tissue seemed to arise close to the dura under the haematoma that formed at operation (fig. 18 a_2). Even when the dura was covered with a polythene film, a bone layer of normal thickness developed, and it arose almost exclusively between the polythene film and the dura (fig. 11 a). It may be assumed that the primary callus originated from the dural cells and was formed beneath the polythene film. The same explanation can be advanced for those cases (Group B) in which the opening was covered with outer periosteum, the result being either good regeneration or regeneration that was better than usual (fig. 9). A claim that the new bone would originate in unspecific connective tissue cells in the dura itself, cannot, however, be disproved with certainty.

The looseness of the osteoblast layer between the dura and the

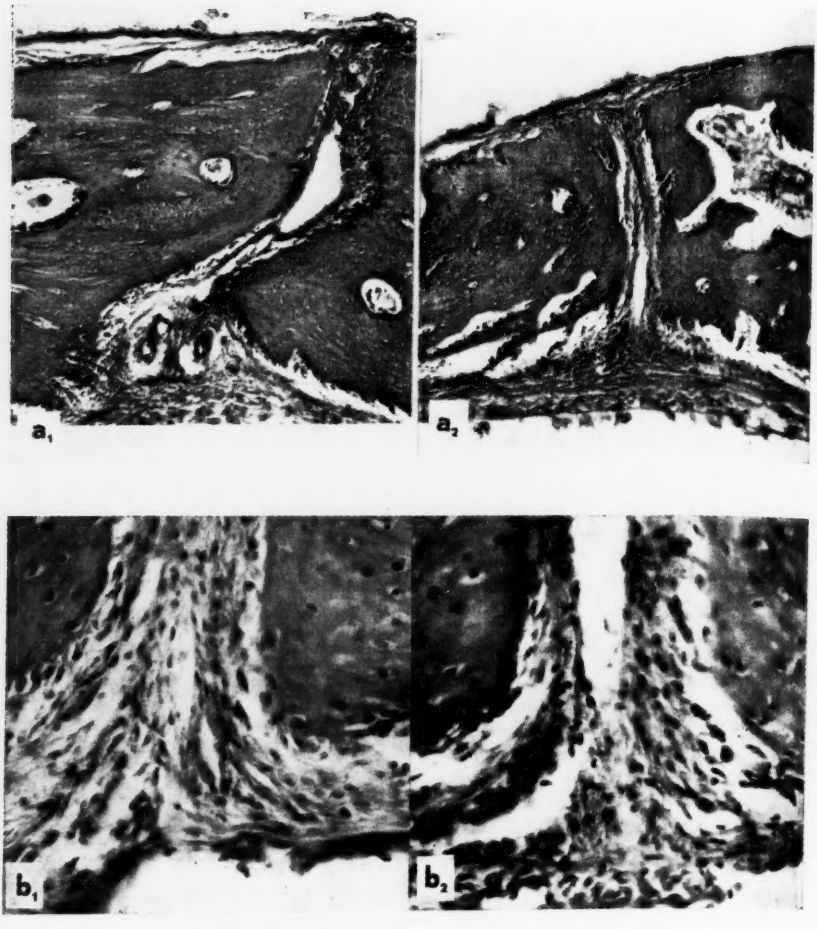


Fig. 22. — Microscopic structure of the normal suture and a secondary suture in the regenerated bone. a_1. The sagittal suture of a 1-month-old rabbit (R 3). a_2. A secondary suture in the same animal ($\times$ 75). b_1. The sagittal suture of a 3½-month-old rabbit (R 32). b_2. Dural side of a secondary suture in the same specimen. $\times$ 300.

bone was mentioned above. It may be assumed that after trephination osteoblasts are unevenly distributed over the dural surface; in places they may even be missing entirely. When the haematoma is resorbed the dural osteoblasts form callus, while at the same time the outer loose connective tissue penetrates into the opening. It can presumably be taken as a fact that once they have become differentiated to the point of being fibroblasts or fibrocytes, connective tissue cells can no longer be transformed into osseous

cells [35, 61, 133]. We may therefore assume that the occupation of areas in the opening in the calvaria by fully differentiated connective tissue leads to inhibition or delay of ossification. In the area of regeneration, competition for space between osteogenic and connective tissue would thus take place. The suture-like formations (fig. 22) which were found in the regenerated area in almost all cases and which did not differ in principle from normal sutures, would thus represent remnants of places in which the callus had been replaced by connective tissue. For these the term »fibrous barrier», applied by Pritchard [127] to normal sutures, could well be used. We may finally assume that such connective tissue, when fully developed and sufficiently firm, constitutes an absolute bar to ossification (see fig. 7 c').

DEPENDANCE OF THE REGENERATIVE CAPACITY OF THE CALVARIA UPON AGE

There has generally been a tendency to correlate the regenerative capacity with the rate of growth. We know that a fracture of the femur consolidates in 1 month in a newborn baby, in 2 months in a 15-year-old and in 3 to 4 months in a 50-year-old person [161]. Thus the younger the individual, the higher the regenerative capacity seems to be. On the other hand, very young tissue may also be more susceptible to permanent injury, particularly during embryonic development, a fact which probably accounts for many congenital anomalies.

Medawar [107] gives the following illustrative example: »Amblyostoma, for example, can regenerate a leg, but it develops without one if the organ-rudiment has been removed from the embryo [63]. Localized trauma or extirpation of the parts of an organ-rudiment produces specific localized defects or abnormalities in the adult organ. If the adult organ is then removed, that which regenerates in its place faithfully reproduces the defects of the original. It appears in these cases that the organism cannot regenerate a structure which has never been formed in its own development [170].»

It appeared from my experiments on cats that a defect made in the calvaria of a 1-week-old kitten became completely ossified within 4 months, while in an adult animal it remained patent to a large extent (fig. 15). Likewise in some adult rabbits, at the site

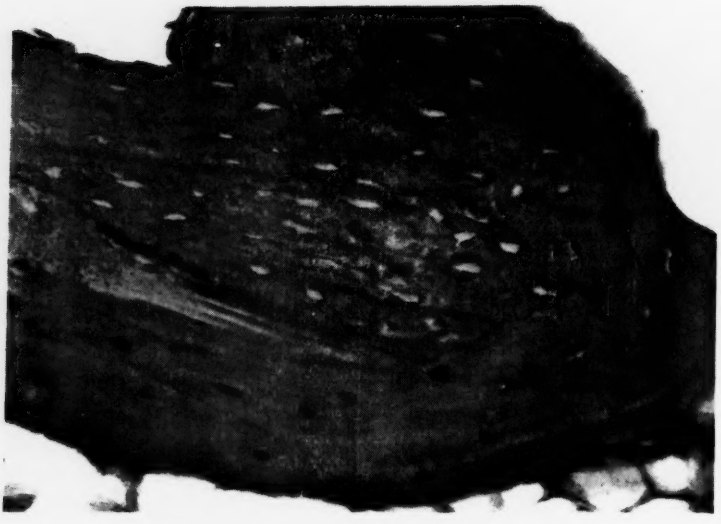


Fig. 23. — Slow remodelling in an adult animal (R 56). At the medial margin of the original trephine opening, non-nucleated bone matrix (centre of picture) can still be seen here and there 6 months after operation within the living new bone tissue. The picture shows a bony process of the inner table towards the diploic space. × 400.

Fig. 24. — The dural surface of the calvaria in rabbits of different ages. *a.* A 4-day-old rabbit. The bone tissue is primitive and the outer and inner tables cannot yet be distinguished from one another. An abundance of active osteoblasts can be seen particularly between the rough bone surfaces and the periosteum (dura or pericranium). × 400. *b.* 1-month-old rabbit. At this stage a diploic space and in it bone marrow are already existent. The osseous structure is largely lamellar. Between the bone and the dura a layer of osteoblasts with large deeply staining nuclei is seen. In the picture these remain at the bone surface but in other places they may be attached to the dura. On the dural surface smaller cells with lighter nuclei (preosteoblasts?) are seen. × 400. *c.* 2½-month-old rabbit (R 23). Here almost the entire osteoblast layer has remained attached to the dura. × 400. *d.* Adult rabbit (R 47). The distinct lamellar structure of the bone and scattered osteocytes with very flat nuclei can be seen. The osteoblast nuclei on the dural surface are likewise very flat and form no clear layer. × 400.

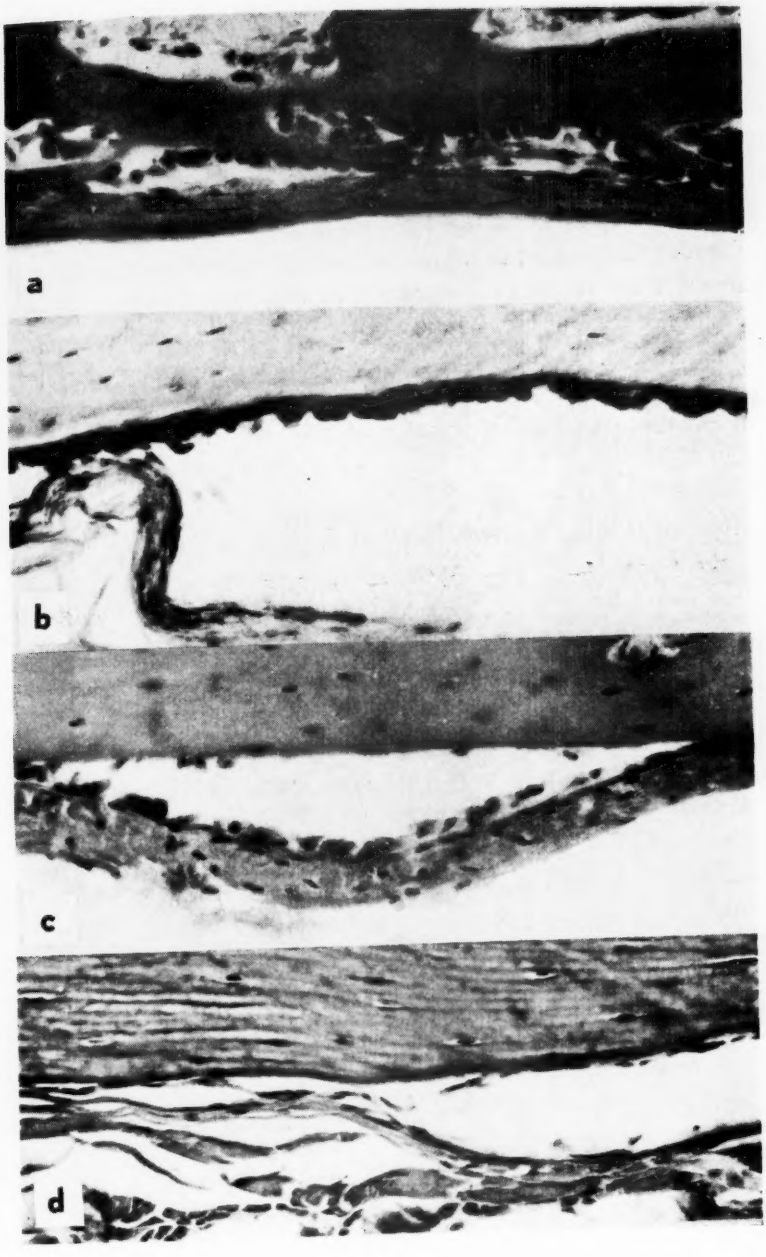


Fig. 24.

of the trephination there remained patent areas covered by connective tissue alone, despite the fact that the dura was macroscopically undamaged (fig. 7). It should be noticed, however, that in adult rabbits a bony cover generally developed over the defect, although, relative to the normal thickness of the calvaria, the new bone remained considerably thinner than in young rabbits. The ossification defects occurring in the youngest group of rabbits (fig. 5) cannot simply be considered a sign of poor regenerative capacity, but must be ascribed to macro- or microscopic damage to the dura. Around these defects regeneration of the bone was usually good. In many of these cases a rupture of the dura could be microscopically demonstrated. The dura of a small rabbit is actually very much thinner than, for instance, that of a cat of the same age. In a young animal the remodelling capacity of the bone is better than in an adult. Microscopic examinations showed that in the youngest rabbits necrotic bone at the margins of the defect would disappear entirely within a month, whereas in adult animals at sites corresponding to the margins of the original defect distinct devitalized bone areas could be seen as much as 6 months after operation (fig. 23).

In a young growing animal the osteogenic layer under the periosteum and thus between the dura and the bone, is strikingly thick and the nuclei there are large and angular (active osteoblasts [128], fig. 24 a and b). In adult animals, in contrast, the osteoblasts form a very thin layer on the surface of the bone and their nuclei are small and flat (fig. 24 d). It seems legitimate to assume that in a young animal the formation of callus is easier and that final regeneration takes place more readily, since there is already a larger supply of osteogenic cells. There is no reason in this connection to try to gain deeper insight into those factors which link together the phenomena of growth and regeneration. It should be mentioned, however, that the growth hormone has been shown to be essential for the regeneration of the calvaria [147].

CONCLUSIONS

1. In rabbits and cats regeneration of varying degree could regularly be observed in trephine openings in the calvaria provided the dura mater had been preserved. The regenerative capacity of the calvarial bones was comparatively good in these animals and obviously considerably better than in man. Since in all mammals, at least, the bones of the cranial vault, despite some notable differences, show strong similarities with regard to their embryogenesis and structure, it is possible that the results of the present investigation may throw some light on the general problem of the regeneration of the calvarial bones.

2. An important feature of this regeneration is the rapid formation of callus within the course of a few days in the trephine opening, which is first covered by a haematoma. In the formation of callus the osteogenic cells which remain on the dural surface must be considered to play an important part, particularly in respect of ossification of the defect. It is obvious that the bone margins and the outer periosteum also contribute to the formation of callus. Connective tissue invading from outside, however, seems to constitute an obstacle to the formation of bone. The new bone first appears in the callus layer as islands and trabeculae surrounded by osteoblasts, in rabbits 5 to 8 days after trephination.

The sutures do not constitute such fixed topographic limits of the calvarial bones as some earlier authors have assumed. True, in rabbits when the bone regenerates, the coronary suture mostly occurs in approximately the correct place but its course is irregular and often asymmetric relative to that of the contralateral side. Four months after operation its configuration in rabbits is still considerably more primitive than normal. The coronary suture does not seem to regenerate in cases in which a polythene film has been placed over the dura in the trephine opening. In the area from

which the bone has been removed numerous secondary sutures also occur, which are probably remnants of areas occupied by connective tissue during the first postoperative days. The microscopic structure of these sutures does not differ essentially from that of a normal suture. Judging from the occurrence of primitive bone tissue and active osteoblasts, it may be presumed that the growth in width of the bone takes place at these sites in the same way as in a normal suture.

3. Regeneration is prevented if the dura mater is excised from the area of the trephine opening. The brain surface will adhere strongly to the outer connective tissue. Adhesion of connective tissue to the brain surface probably has a directly inhibitory effect upon the regeneration process. Such adhesion can for the most part be avoided by covering the dural defect with a polythene film or with periosteum, for instance; normal regeneration cannot be restored in this way, however.

The dura itself is able to produce bone, since isolated bone islands may occur on its surface and since bone is formed even when the margins of the defect have been isolated. If the dural surface exposed at trephination is covered with a polythene film, the new bone is principally formed between the film and the dura.

Somewhat more bone is regenerated at the margins of the defect than at the centre, particularly in the lateral portion. Trephination will produce a narrow strip of dead bone at the edges of the defect which will slowly be replaced by new bone.

It can seems obvious that the pericranium, too, plays a part in the formation of new bone. However, in experiments in which the pericranium is freed from the bone and replaced to cover the defect, its bone-forming capacity seems much lower than that of the dura.

4. In the human calvaria ossification of defects is probably very different at different ages. In rabbits this variation is not so pronounced. Because of the great risk of operative injury to the very thin dura, regeneration is uncertain in quite young rabbits, the osteogenic layer being easily damaged, not only macroscopically but also microscopically. In young 1½- to 3-month-old rabbits, however, regeneration is good. In adult rabbits comparatively good regeneration can sometimes be observed, although the new bone layer is thin compared with the normal bone and only slowly grows in thickness. In adult rabbits clear ossification defects often

persist and these heal very slowly, if at all. Furthermore, the younger the individual the quicker is the remodelling of the margins of the defect, which includes substitution of the necrotic strip of bone at the margin with living bone. In cats the regenerative capacity of the calvaria seems to be more clearly correlated with age than in rabbits. The higher regenerative capacity of the calvaria in young as compared with adult individuals should be considered to result from the thicker osteogenic cell layer lining the surfaces of the growing bone in the former.

SUMMARY

The author first surveys the information on the regeneration of the bones of the cranial vault obtained from studies on prehistoric skulls, clinical observations and earlier experimental investigations. He finds that according to prevailing opinion an opening trephined into the skull of an adult human individual remains patent, while in a child, and particularly in an infant, such a defect may ossify remarkably well.

The experimental series of the present investigation comprises animals of different ages, from newborn to adult: 83 rabbits and 8 cats. In the majority of cases the operative method was first to remove the outer periosteum (pericranium) from the trephination area and then freeing it carefully from the dura mater to resect bone from a comparatively large area of the parietal and frontal bones (Group A). The part played by the pericranium in regeneration was studied in other experiments in which it was temporarily freed from the bone as a lateral flap for the duration of the operation and replaced over the defect (Group B). The influence of the dura on the course of regeneration was studied by removing it entirely from the area of the defect in some animals (Group E). In some cases the defect in the dura was covered with a thin polythene film or with a flap of the outer periosteum. In one group the dural surface exposed at trephination was covered with a thin polythene film which was pushed a little under the bone margins (Group C). In some other cases the margins of the defect were covered with narrow strips of polythene to isolate the exposed dural area from the bone (Group D).

The animals were killed after periods of varying length, and the cranial vaults were sawn off and studied macroscopically. With a biological X-ray apparatus detailed pictures were obtained of the bone specimens and these were then used in conjunction with microscopic preparations for a study of the regenerative changes.

Regeneration could be observed in the trephine defect of the

calvaria of experimental animals, provided the dura mater had been preserved. In rabbits the removed bone was replaced within a few days by a callus plate fusing with the dura and in this plate new bone began to develop from the fifth postoperative day on in the shape of islands and trabeculae surrounded by active osteoblasts. The author believes that the rapid division of the osteogenic cells persisting on the surface of the dura plays the most important rôle in the formation of the callus plate. It is to be assumed that the osteogenic cell layer on the dural surface has been abolished in places as a result of mechanical factors, the osteoblasts adhering to the surface of the removed bone, for instance. These sites are invaded by connective tissue from outside which later appears in the new bone in the form of connective tissue suture formations. These secondary sutures are microscopically, and obviously also to some extent functionally, similar in principle to normal sutures. Such a suture is constantly formed at the site of the coronary suture in the area of trephination. On the other hand, the coronary suture did not seem to regenerate in those cases in which a polythene film had been placed over the exposed dura.

If the dura mater is removed from the trephine area, regeneration of the bone is prevented and the brain surface adheres firmly to the outer connective tissue. In the course of regeneration the dura is of fundamental importance and is of itself capable of producing bone. The margins of the defect and the pericranium also take part in the formation of bone, although to a lesser extent.

In cats it was possible to demonstrate a correlation similar to that obviously existing in man, between the age and regeneration of the defect in the calvaria. In rabbits of the youngest age group (under 6 weeks old) regeneration was usually incomplete, obviously as a result of operative injury to the dura or its osteogenic layer. In 6- to 8-week-old rabbits very good regeneration was observed. In adult rabbits a new bone layer developed in the defect in many cases but it was proportionally very thin as compared with the group of young rabbits. Actual ossification defects also often persisted in adult rabbits. The older the individual, the slower was the remodelling of the margins of the defect, which was apparent as a resorption of the non-nucleated bone matrix from the margins and as a disappearance of the contours of the defect in the radiogram.

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